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Genetic indicators for disease resilience in pigs

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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ABSTRACT

Infectious swine diseases have the potential to decimate the health and productivity of swine farms. One of the most economically concerning diseases is caused by the Porcine Reproductive and Respiratory Syndrome (PRRS) virus. While swine producers can implement vaccines, medications, or antibiotics and antiviral drugs, many infectious pathogens such as the PRRS virus have shown these strategies to be ineffective. One complimentary strategy would be to select pigs for increased disease resistance or resilience, where disease resilience is defined as an animal's ability to maintain performance when infected. However, the elite populations that are used for genetic improvement are typically kept in high health conditions, making it difficult and impractical for swine breeders to use phenotypic selection in an environment with exposure to disease to select for increased disease resilience. Previous research has shown that host response to PRRS virus infection has a sizable genetic component and revealed a Quantitative Trait Locus (QTL) for host response to PRRS virus infection on *Sus Scrofa* Chromosome (SSC) 4. A putative causative mutation in the GBP5 gene was identified for this QTL. This mutation was determined to be in complete linkage disequilibrium with the single nucleotide polymorphism (SNP) WUR10000125 (WUR) that was included on commercial SNP panels. However, this was based on data from one genetic source.

The overall objective of this thesis was to determine if the WUR SNP and phenotypes obtained from in-vitro mitogen stimulation assays (MSA) of peripheral blood mononuclear cells (PBMCs) from young healthy nursery pigs can be used as genetic indicators to select for disease resilience. The two requirements for a genetic indicator are that it must be heritable and have a sizeable genetic correlation with the trait of interest, in this case, disease resilience. Data from experimental PRRS virus infection trials from the PRRS Host Genetics Consortium (PHGC) and

a polymicrobial Natural Disease Challenge Model (NDCM) of grow-finish pigs were used to address these objectives.

Data and SNP genotypes, including for the WUR SNP and the putative causative mutation in the GBP5 gene, were available on 1414 pigs from eight PHGC trials of ~200 commercial crossbred nursery pigs per trial from six unrelated populations. Results showed that the WUR and GBP5 SNPs were not in complete linkage disequilibrium ($r^2 = 0.94$). Discordant genotypes were determined to be the result of recombination, rather than genotyping errors. Although it was previously speculated that the GBP5 gene is a major gene responsible for host response to PRRS, there were small but non-significant differences between the effect of GBP5 and WUR on PRRS viral load and weight gain post-infection. These results indicate that either GBP5 or the WUR SNP can be used for marker-assisted selection to increase resistance to PRRS.

In the NDCM, data from 3139 crossbred nursery barrows that were genotyped using a 650 K SNP Panel (Affymetrix) were used. The 650 K panel included the WUR SNP but not the GBP5 SNP. In the NDCM, pigs were entered through a batch system of 60 or 75 pigs per batch into a facility that was seeded with multiple infectious pathogens, including PRRS, to maximize the expression of disease resilience. Disease resilience traits, including growth, feed intake, and treatment and mortality rates were recorded. Based on these data, it was determined that the favorable G allele for the WUR SNP was significantly associated with greater average daily gain ($p=0.02$) and lower numbers of treatments in the challenge nursery ($p=0.05$) and across the challenge nursery and finisher ($p=0.01$), establishing the effect of the SSC4 QTL on resilience to a polymicrobial disease challenge.

For the MSAs, PBMCs were isolated from blood samples of 882 pigs from 19 batches of the NDCM, taken at 27 or 35 days of age and prior to their entry in the disease challenge. For the MSAs, PBMCs were stimulated with five unique mitogens: Concanavalin A (Con A), Phytohemagglutinin (PHA), Poke Weed Mitogen (PWM), Lipopolysaccharide (LPS), and Phorbol Myristate Acetate (PMA), and evaluated for counts of proliferated cells after 48, 72, and 96 hours compared to unstimulated samples (restcount). Proliferated cell counts were adjusted for restcount in two ways: 1) by dividing the average cell count of the stimulated wells by the average cell count of the non-stimulated wells, to compute a Blastogenic Index Score (BIS), and 2) by including the average cell count of the non-stimulated wells as a covariate in the model for analysis of the average cell count of the stimulated wells. Data on BIS and stimulated means at each time point were analyzed separately for each mitogen. For pigs that had data at all three time points for a mitogen, data across these time points were incorporated into a single phenotype called the Area Under the Curve (AUC). Differences between pairs of time points for a given mitogen (delta = 72 – 48 hrs, 96 – 72 hrs, and 96 - 48 hrs) were also analyzed as phenotypes. Genetic parameters (heritabilities and genetic correlations) were estimated for the MSA phenotypes. In general, MSA phenotypes based on BIS versus stimulated means adjusted for restcount had similar estimates of genetic parameters. Heritability estimates for the Con A, PHA, and PMA MSA phenotypes were moderate, ranging from 0.13 $\pm$0.09 to 0.37 $\pm$0.10 for Con A, from 0.10 $\pm$0.07 to 0.34 $\pm$0.09 for PHA, and from 0.05 $\pm$0.06 to 0.30 $\pm$0.10 for PMA. Heritability estimates for the PWM and LPS MSA phenotypes were low, ranging from 0.00 $\pm$0.00 to 0.15 $\pm$0.09. Disease-related phenotypes collected on these same pigs in the NDCM were then used to estimate genetic correlations of the MSA phenotypes with disease resilience phenotypes. Phenotypic correlations between MSA and disease resilience phenotypes were low.

Phenotypes derived from the Con A, PHA, and PMA MSAs, however, had moderately high estimates of genetic correlations with several disease resilience traits, although none were significantly different from zero due to large standard errors. However, genetic correlation estimates were generally in the expected direction, with pigs with higher MSA response having better resilience at the genetic level. Overall, Con A presented itself as the most promising mitogen to use as a genetic indicator for disease resilience, although further studies are recommended to validate its potential and to determine the ideal time point or MSA phenotype to use.

In conclusion, the use of a genetic indicator to indirectly select for increased disease resilience in swine is a viable approach. The two indicators investigated in this thesis, i.e. genotype at an SNP on chromosome 4 and results of an in vitro mitogen stimulation assay on immune cells derived from the blood of young healthy piglets, are suitable genetic indicators for disease resilience to a polymicrobial disease challenge.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Genetic improvement of swine has resulted in large increases in several desired production traits in the US pork industry. The impact of infectious swine diseases, however, persists as an economic problem. One such pathogenic disease is Porcine Reproductive and Respiratory Syndrome (PRRS), caused by the PRRS virus, which increases reproductive failures in sows and mortality in younger pigs. One study estimated that the PRRS virus costs the US Pork industry 665 million dollars each year (Holtkamp et al., 2013). Due to the severity of this disease, the National Pork Board (NPB) funded the PRRS Host Genetics Consortium (PHGC) in 2007, a community of PRRS researchers collaborating with industry to develop strategies to combat PRRS by increasing host response to PRRS virus infection (Lunney et al., 2011).

While selection for production traits continues to increase revenue for producers, one reason why swine diseases linger as an economical problem in the pork industry may be due to the "Resource Allocation Theory" (Rauw, 2007). Based on this theory, animals that are selected for increased production efficiency have genetic pressure to allocate energy resources to those traits, leaving less energy to be allocated to other energy-demanding traits, for example, the immune response to a pathogenic threat. Therefore, as animals in the market are more and more selected for production efficiency, there is a dire need to also consider their ability to fend off disease. A parallel concept is the term "disease resilience" which describes the productiveness of an animal during the course of an infection (Albers et al., 1987; Doeschl-Wilson et al., 2012; Mulder and Rashidi, 2017). Fortunately, in principle, selection is not limited to just production

traits and can also include selection on disease resilience traits. However, several issues need to be overcome to improve disease resilience in commercial swine through genetic selection.

To understand the challenges of selecting for disease-related traits in the swine industry, the foundations of the swine industry must first be discussed. There are large genetic and phenotypic differences between pigs from different lines and breeds. By crossing these parental lines, producers can take advantage of each breed's specialties, as well as heterosis (Fragomeni et al., 2016). To exploit the economic benefits of heterosis, the structure of the pork industry in the United States has evolved from backyard pigs into what can be best described as a three-tiered pyramid structure, as illustrated in Figure 1.1.

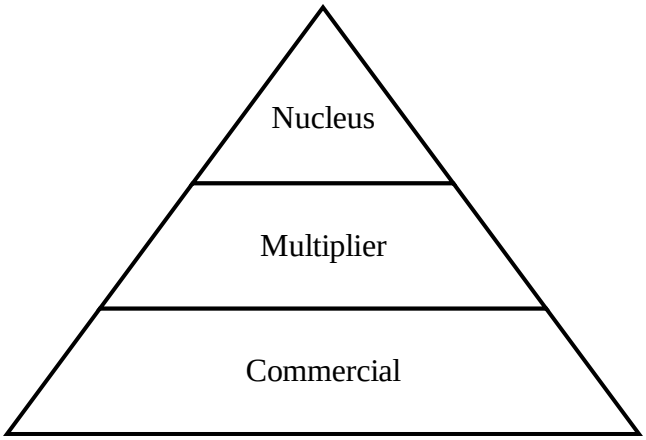

Figure 1.1: Structure of the US Swine Industry.

The very top tier consists of the nucleus populations, elite groups of pigs of the parental breeds or lines that are intensely and meticulously selected for specific production traits. These pigs tend to be from purebred breeds or lines. Some of the specific dam and sire lines utilized as nucleus populations are from the Landrace, Yorkshire, and Duroc breeds. The Landrace,

Yorkshire, and Large White are considered “maternal breeds”, as their lines are recognized and selected for traits such as litter size and weaning weight (Bidanel et al., 1994). On the other hand, the Duroc and Hampshire are considered “paternal breeds”, as these breeds are recognized for traits such as meat color, carcass size, and other desirable meat quality traits (Nowachowicz et al., 2000).

Nucleus herds are typically of limited size (500 to 2000 sows). For these herds to provide sufficient breeding animals to meet the needs of commercial pork production, one or more multiplier levels are included, in particular on the maternal side. The multipliers have a larger population size than the nucleus and exploit the advantages of crossing the purebred lines, in particular, to produce F1 females on the maternal side by crossing the Landrace and the Yorkshire breeds, to capitalize on maternal heterosis (Johnson et al., 1975).

The pork that consumers purchase is not from either the nucleus or the multiplier pigs, but the commercial pigs. The base of the three-tiered pyramid is occupied by the commercial swine herd. These swine are the offspring of the multiplier pigs and have the largest population size among the three tiers. The pork that consumers purchase is from the commercial swine herd, leaving great reason for them to be concerned about the welfare and the health of commercial swine.

Overall, the pyramid structure allows genetics from the nucleus herd to cascade into the commercial herd. Each tier consists of different sized populations, housing environments, management techniques, and risk of disease. In particular, because of their value, nucleus herds are kept under high biosecurity units to ensure the protection of these pigs from infectious pathogens that are commonly found in commercial barns. In addition, international or interstate

transport of swine germplasm can require a health certificate. Thus, when disease enters a nucleus herd, the possibility of germplasm being sold from this herd could be eliminated, a huge financial detriment for swine companies that commercialize high-quality boar sperm and animals.

Due to the multi-tiered pyramid structure of the pork industry, to create genetic change in the commercial swine herd, genetic change must be implemented upstream in the nucleus tier. In principle, a disease challenge could be performed in the nucleus herd to observe and allocate the animals that should be used in selecting for higher disease-related traits. However, this is problematic for two main reasons. First, it is not cost-effective to put the nucleus herd through a disease challenge because all of them may die or permanently lose their production ability due to disease. Second, the presence of a disease in the nucleus would prevent germplasm to be marketed from this population. Instead, the nucleus herds must be kept as healthy and intact as possible to provide the desired genetics for the lower tiers in the pyramid. In order to circumnavigate these two problems, the use of genetic indicators for disease-related traits could be considered for selection in the nucleus.

Genetic indicators are traits that can be used for selection to genetically improve a target trait, instead of selecting directly on the target trait (Oliver et al., 1958). Genetic indicator traits are used when the target trait is difficult to select for directly because the desired trait may not be observed, such as the maternal ability of male hogs, or if traits are measured too late, such as carcass traits. There are multiple requirements for a genetic indicator to be effective but, specifically, they must be (1) heritable and (2) the genetic indicator must be genetically correlated with the target trait. The underlying genetic mechanism that allows for the latter is

pleiotropy, in which a given gene influences two or more phenotypes (Paaby et al., 2013). Historically, farmers would use selective breeding (artificial selection) of animals, choosing animals based on observable phenotypes that indicated favorable traits (Wykes, 2004). More recently, the use of genetic markers has been implemented as a tool to be used in marker assisted selection. This strategy relies on genetic markers, which are genes or variations in DNA sequences that can be used to identify genetic characteristics of an organism that are associated with a phenotype. Commonly used genetic markers include restriction fragment length polymorphisms and single nucleotide polymorphisms (SNPs) (Collar et al., 2008).

The first study in this thesis explored the effect of genotype at genetic marker WUR10000125 (WUR) on performance in a polymicrobial disease challenge to determine if genotype at the WUR marker could be a possible indicator for disease resilience. Previous studies have shown that pigs with the favorable genotype at this genetic marker had greater resistance and resilience to a PRRS disease challenge (Boddicker et al. 2012) and that this genetic marker was in complete linkage disequilibrium with the putative causative mutation for host resistance to PRRS in the nearby GBP5 gene (Koltes et al. 2015). Thus, the objectives of this study were to 1) investigate the relationship of genotype at the WUR SNP with genotype at the putative causative mutation in the GBP5 gene, 2) compare the association of the WUR versus the GBP5 SNP with host response to PRRS virus infection, and 3) determine whether the WUR SNP is also associated with resilience to a polymicrobial disease challenge.

The second study in this thesis used mitogens as an immune cell stimulant in vitro to determine if the rate of peripheral blood mononuclear cell (PBMC) proliferation in stimulated blood from young healthy piglets can be used as a genetic indicator for disease resilience. As

mentioned earlier, genetic indicators must be heritable and genetically correlated to a target trait. Thus, the objectives of this study were to determine estimates of genetic parameters of these mitogen stimulation assays (MSAs), i.e. the heritability of PBMC proliferation rate following stimulation with various mitogens at different time points after stimulation, and the genetic correlations of these proliferation rates with disease resilience traits.

Thesis Organization

The second chapter of this thesis is a review of the current literature related to genetic selection for disease resilience in growing pigs. Topics discussed include the mammalian immune response to pathogens, the impact of PRRS and other swine pathogens on disease resilience, measurement of disease resilience phenotypes using in vitro assays, and the genetics of disease and resilience. The third chapter reports on a study of the analysis of differences in disease resilience phenotypes based on genotype at the WUR marker, to determine if the WUR genotype can be used as a genetic indicator to resilience under a polymicrobial disease challenge. The fourth chapter describes results from mitogen stimulation assays (MSAs) in blood collected from innate naïve piglets, to determine whether they can be used as genetic indicators for disease resilience. Chapter 5 discusses the key results obtained from these two studies and how results can contribute to the improvement of disease resilience in commercial growing pigs. Chapter 3 is formatted for the journal Livestock Science and Chapter 4 is formatted for the Journal of Animal Science. All data were obtained from the PHGC and the NDCM, which was implemented at the Centre de développement du porc du Québec (CDPQ) in Quebec, Canada.

For the studies described in Chapter 3, I (Ryan Jeon) conducted the statistical and bioinformatic analyses of the effect of the WUR genotype on disease resilience, while Dr. Qian.

Dong conducted the analyses of the PHGC data. Jian Mark Cheng and Dr. Austin Putz from Iowa State University created the dataset, consisting of all disease resilience phenotypes used for analysis and provided a polished genotype file that included the WUR marker. The disease resilience data were collected as part of a large-scale natural disease challenge study that was held in facilities at CDPQ. Interpretation of results from the PHGC trials was done by Qian Dong and me. The interpretation of results from CDPQ was done by me. The results in this chapter were written and prepared by me.

For the studies described in Chapter 4, I conducted the statistical analyses on exploring the prospect of using results from MSAs as genetic indicators for disease resilience in growing pigs. The MSA assays were designed by Dr. Jenny Phipps from Metadis Inc. and were completed and compiled by Dr. Caroline Gilbert from the University of Laval and by Dr. John Harding from the University of Saskatchewan. The methods section regarding the mitogen stimulation assay protocol was written by Dr. Caroline Gilbert and Dr. John Harding. The remaining methods section was written by me. The rest of this chapter was written and prepared by me. Chapter 5 was prepared by me.

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CHAPTER 2. LITERATURE REVIEW

Quantitative Genetics

The study of animal genetics was born from an intent for animal breeders to acquire a better understanding of the inheritance of economically desired traits in their flocks, herds, and litters. The value of an animal as a parent can be measured by the expression of an animal's genetics into phenotypes. These phenotypes can be visible or non-visible and can be categorized as either quantitative or qualitative phenotypes. Qualitative phenotypes are often visible traits that are measured subjectively and divided into categories. These traits are often controlled by one or a few genes. However, there are many polygenic qualitative genes, such as those affecting the health status of an animal (healthy or sick). On the other hand, quantitative traits show continuous expression and are numerically measured. These traits are controlled by many genes, causing complexity in determining the contribution of each gene on a given quantitative trait. Phenotype is not limited to just the genetics, however. The environment an animal is in will also influence the phenotype. An animal with superior genetics will not realize its full potential if the environment is not conducive to growth. For instance, an animal heavily selected for production traits may not reach an expected level of production, if the environment has high disease pressure.

An animal's phenotype is determined by genetics and environment, however, only genetics is inherited. The definition of heritability from a statistical perspective is the proportion of observed variance for the trait that can be attributed to inherited genetic factors, rather than the environment (Wray and Visscher, 2008). In other words, it is the proportion of variation in a population of a given phenotype that is due to genetic variation. Heritability runs from a scale of

0 to 1, where high heritability is considered to be greater 0.4, while traits that are lowly heritable range from 0 to 0.2. The heritability of a trait is not concrete and can differ between species, lines, and given enough time, even within the same population.

Heritability is fundamentally important to the accuracy and efficiency of selection because it can be used as a measure of how well the performance of a particular trait from the offspring will resemble the performance of its parents. Take for instance, the most rudimentary form of selection, phenotypic selection. In this method, animals are selected solely based on their own observable phenotype. One particular example would be to select parents based on their own resilience to disease. If the heritability of resilience to disease was high, then the parent's performance would be a great indicator of the offspring's performance. On the contrary, if heritability was low, then the offspring would be unlikely to resemble their parents for this trait. Modern selection these days utilizes more information than just the own observable phenotype, for instance phenotypes of relatives and, more recently, genotyping data. However, this example delineates the importance of heritability in selection.

There are many methods to improve a population of animals (inbreeding, line-breeding, outcrossing), but there are two key components that are central to all of these. The first is selection, in which animal breeders must choose which animals become parents, as well as how long each animal will stay in the breeding program. The second is non-random mating, in which animal breeders must choose the male and female parents to mate to create offspring. Until the 1970s, the selection of the animals was determined from an animal's observable phenotype. It was only until the arrival of genetic markers that the possibility for selection based on genotype, rather than observable phenotype was attained.

A Brief History of Genetic Markers

Genetic variation describes differences in DNA sequences between individuals in a population (Barton and Keightley, 2002). These differences are due to polymorphisms, or changes in the DNA sequence that can create variation of a trait in a population. Each individual carries two copies of nucleotide sequence at each part of the genome (pairs of chromosomes), but not every nucleotide sequence will be identical. The simplest polymorphism involves variation at one base pair, for instance, a guanine substituted for a cytosine. These are called single nucleotide polymorphisms (SNP) and are the most common types of polymorphisms observed (Teama, 2018).

DNA polymorphisms can be used as markers identify and locate quantitative trait loci (QTL). QTL are regions on the genome that are found to be statistically associated with the variation in phenotype between individuals at a quantitative trait, which can be used to find candidate genes responsible for trait variation.

Markers can be separated into two classes: biochemical and molecular. Biochemical markers represent variation at the protein level, e.g., differences in the form or function of proteins, while molecular markers represent variation at the DNA level, for instance, base pair changes or repeated sequences. The next sections will introduce some of the biochemical and molecular markers that were commonly used to find the underlying genetic basis of trait variation.

Alloenzymes: The first markers used were alloenzymes. Alloenzymes are biochemical markers and can be described as allelic variants of enzymes (Schlötterer, 2004). If an amino acid substitution in DNA causes changes to a protein, this can be measured by using native gel

electrophoresis. This allows visualization of polymorphisms through different colored bands that change based on the charge and size of the protein variant. Several studies have used alloenzymes to study genetic variation in a wide variety of animals, such as humans, Drosophila, and the wild boar. The Lang et al., (2000) found a potential genetic marker in the wild boar that was associated to resistance against swine fever by utilizing alloenzymes. They determined that by comparing enzyme polymorphisms before and after a devastating swine fever, the allele frequency at the phosphoglucomutase locus (PGM-2) changed substantially, indicating evidence that animals with a favorable allele at this locus may be more resistant to swine fever.

Alloenzymes are still used today in populations with large sample sizes, as historically they were one of the most cost-efficient genetic markers. However, the biggest limitation with alloenzymes is that they have limited coverage because the number of informative loci is small. Another limitation is that alloenzymes measure the product of a polymorphism, not the DNA itself, meaning it is difficult to know the number and location of mutations that explain the polymorphism. Last, the procedure can be laborious and with the advent of DNA sequencing technology, the use of alloenzymes has dwindled (Schlötterer, 2004).

Restriction Fragment Length Polymorphisms (RFLPs): Restriction enzymes were the first molecular, or DNA level markers, to be used. The use of RFLP's involve the use of a group of enzymes that search for a specific sequence of nucleotides, also known as restriction sites, and cleave fragments of DNA near these sites. This can be exploited to explore polymorphisms in DNA. If a nucleotide base pair has been switched to another, the restriction enzyme will no longer recognize the site and will not cleave the fragment. The general procedure is to use a specific restriction enzyme to cleave DNA wherever the specified restriction site is located in the genome. These fragments can be separated and visualized through the use of gel electrophoresis

and Southern blotting. Polymorphisms can be detected through this technique if the size of one fragment differs from the others (Beckmann and Soller, 1983). Several studies using RFLPs have been performed to explore genetic diversity in swine populations. Yu et al. (1994) used RFLP's to study the association between economic traits and the *PIT-1* gene, a pituitary transcription factor responsible for the activation of growth hormone in swine. Using RFLP's, this study found associations between polymorphisms in the *PIT-1* gene with carcass and performance traits, suggesting that PIT-1 was a candidate gene for a QTL for this trait. The biggest limitation of RFLPs is that this molecular technique can take a long time and requires several different cumbersome steps. Much like the use of alloenzymes, the laborious steps required for the use of RFLPs became overshadowed by the advent of PCR markers (Schlötterer, 2004).

PCR Markers: In the 1980s, a DNA amplification technique called Polymerase Chain Reaction (PCR) revolutionized DNA markers even further. PCR is a common tool that can make microgram quantities of billions of copies of a particular DNA segment by using primers (Singh and Singh, 2015). By amplifying a sample of a DNA segment, detection of polymorphisms is possible through the using primers as a marker. One of the marker types that utilizes PCR is the microsatellite. Microsatellites are repeats of DNA motifs and are highly polymorphic due to frequent mutations in these repeats. Microsatellites are abundant across the genome. These features of microsatellites allow scientists to create a DNA fingerprint that is unique to every individual (Nader et al., 2016). Analysis of DNA fingerprinting has led to several commercial applications, including paternity testing and identifying biological evidence in crime scenes. (Jobling and Gill, 2004). In a study specific to swine, microsatellites were used to study genetic variation between five populations of the Taihu pig (Fan et al., 2002). Using 27 microsatellites, they were able to confirm that Chinese pig breeds (Shawutou, Jiangquhai, and Dongchuan) and

European breeds (Large White, Landrace, and Duroc) belong to two divergent groups. While microsatellites have been successfully implemented in a wide variety of genetic and commercial applications, their biggest limitations were their high implementation cost and difficulties with studying unconventional model organisms.

Single Nucleotide Polymorphisms (SNPs): Alloenzymes, RFLPs, and PCR markers have been very useful in differentiating between allelic variants in proteins and DNA. The rise of DNA sequencing has, however, facilitated the discovery of single nucleotide polymorphisms (SNPs). SNPs can be defined simply as a polymorphism at a single base nucleotide at a specific position in the genome. SNPs can be located in non-coding and coding regions of the genome (SNP FAQ Archive, 2005). While the non-coding region does not produce protein through translation, products of non-coding regions or the sequences themselves have been found to participate in regulating transcription and translation. One study even indicated that SNPs in non-coding regions are associated with various common diseases, such as cancer (Chu and Wei, 2019). SNPs in non-coding regions that are found to be associated with a trait can also be used as a marker. They can be used for QTL detection, which is reliant on linkage disequilibrium (LD) between markers and a QTL, which is defined as the non-random association of alleles at different loci on the same chromosome in a population. (Mackay and Powell, 2007)

SNPs that are located in protein-coding regions can be further divided into synonymous and non-synonymous SNPs. A synonymous SNP is one that does not change or affect the protein produced. This is because there are 64 possible combinations of the four nucleotides in a three-base-pair codon ($4_3 = 64$) but there are only 20 encoded amino acids plus one codon for the termination of translation (Avise, 2014). This means that for many amino acids, different codons yield the same amino acid. Since SNPs are a substitution of a single base pair, translation of

different mRNA may incorporate the same amino acid, creating no change in the polypeptide chain. For this reason, synonymous SNPs are called silent mutations. Codon usage bias, which is defined as the differences in the frequencies of the occurrence of synonymous codons in coding DNA (Grantham et al., 1980), has been observed in several organisms, including humans (Hunt et al., 2009). While there are many synonymous codons that code for the same amino acid, there are optimal codons that an organism prefers for efficient translation of mRNA. This explains the potentially harmful effects of many silent mutations. Hunt et al. (2009) observed that silent mutations can affect the response of patients to various medications.

Non-synonymous SNPs are polymorphisms that can directly change the amino acid sequence of a protein. This is the case where a single base pair change in a codon alters the amino acid that it encodes for, thus altering the structure and function of a protein (Hunt et al., 2009). There are two types of non-synonymous SNPs: missense and nonsense. A missense SNP is a point mutation that changes the amino acid that the codon encodes for (Pal and Moult, 2015). Proteins are highly sensitive to these changes on the tertiary level, as the structure of a protein is heavily reliant on the spatial organization of hydrophobic and hydrophilic amino acids (Camiloni et al., 2016). Incorporating a different amino acid will often result in changes to protein folding, which can lead to the onset of genetic diseases, such as Crohn's disease (Collij, 2019). A nonsense SNP on the other hand is a point mutation that creates a premature stop codon, terminating translation (Chu and Wei, 2019). This creates a truncated protein, which results in an incomplete and often nonfunctional protein product.

The use of SNPs is advantageous over the use of other polymorphisms to measure DNA-level variation because of their frequency and their ease of use. SNPs are abundant in the genome of animals, occurring on average once every 300 base pairs in the human genome

(Nelson et al., 2004). To date, millions of SNPs have been documented in humans, and their dense distribution across the genome allows them to be effectively used in genome-wide association studies (GWAS) to uncover genes associated with diseases. SNPs are often measured through the use of SNP panels, which are reliable tools due to them having a generally low genotype calling error rate (Ranade et al., 2001). Modern technology has facilitated high-throughput analyses, enabling large scale population-level studies using a very large number of SNPs (Vignal et al., 2002). Also, SNPs are bi-allelic, which means there are only two allelic variants. This genetic characteristic of SNPs allows assaying of SNPs to be relatively easier than other DNA polymorphisms since the frequencies of each of the two alleles have a two-component ratio (Foster et al., 2011). However, this also creates a limitation for SNPs because each marker will provide a relatively small amount of information. Thus, a considerably larger number of SNPs are required to identify associations with disease compared to using a more polymorphic genetic marker such as microsatellites. Another issue is that many high-throughput methods require expensive equipment. For non-model organisms without conventional SNP panels, the costs of genotyping and development of markers can be a limiting factor in the use of SNPs. Another limitation is that SNP discovery can introduce ascertainment bias (Nielsen et al., 2000). This bias occurs in the development of SNP panels where SNPs with high frequency are often chosen over SNPs that are rare or if individuals are non-randomly selected. This bias can create problems when making inferences or associations with diseases if a rare SNP that was actually associated with a disease, was not included in the panel. However, ascertainment bias is not unique to just the use of SNP's and is a problem in all experimental studies using a non-random population. Despite these limitations, the benefits of SNPs far outweigh these

disadvantages. By utilizing SNPs, animal breeders can take advantage of LD in the form of genetic markers.

Genetic Indicators

A genetic indicator is a trait that is genetically correlated to a trait of interest. Several factors determine the success of a genetic indicator, in particular the following two requirements. First, the genetic indicator must be heritable. As previously discussed, if a trait is not heritable, selection on that trait will not result in genetic change in the population. The second requirement is that there must be a strong genetic correlation between the genetic indicator and the target trait. The presence of a genetic correlation between two traits usually implies that there is a pleiotropic relationship between the two traits, where the same genes affect both traits, in this case, the genetic indicator and the target trait. Both the genetic indicator and the trait of interest must also be measurable to be able to calculate a genetic correlation. It is also important to have a data acquisition system enabled to measure the wide phenotypic variation of both the genetic indicator trait and the trait of interest. This system should allow for the measurement of a genetic indicator trait on selection candidates, such as animals belonging to the nucleus herd. The recording cost should be low for a genetic indicator to be financially viable to a producer or a breeder. Last, it would be ideal for the genetic indicator to be measured early in life because they can provide information about the animal's expected productivity, allowing producers or breeders to make decisions on which animals to keep. The use of genetic indicators that are measured early in life can reduce generation intervals since young animals can be used for selection as accurately as older animals (Schaeffer, 2006).

Major Swine Diseases

A swine barn often harbors several multiple pathogens, ranging from bacteria to viruses to parasitic organisms. Several major swine pathogens play an influential role in the swine industry, including Porcine Reproductive and Respiratory Syndrome virus, Porcine Circovirus, Porcine Rotavirus, Porcine Parvovirus, as well as pathogens that cause coccidiosis and mastitis. In this section, three of these diseases will be discussed due to their relevance in the swine industry and because they were examples of viruses that were used the studies described in Chapters 3 and 4 of this thesis.

Porcine Reproductive and Respiratory Syndrome Virus: Porcine Reproductive and Respiratory Syndrome (PRRS) virus is an Arterivirus that costs the US Swine industry over 664 million dollars each year (Holtkamp, 2013). In pregnant sows, the morbid impact of PRRS can cause stillborn piglets, mummified corpses, and abortions. Piglets that do survive past parturition often face a high rate of pre-weaning piglet mortality. PRRS is not limited to just sows and piglets, as the PRRS virus has been isolated before in semen from infected boars. Also, PRRS can cause severe respiratory problems in all pigs, resulting in weak and frail pigs. These pigs often do not eat and are unable to gain weight and thus, are unable to quickly reach market weight (Bøtner, 1997). In a swine production system, these sets of symptoms are a heavy economic detriment to the US swine industry.

Detection of PRRS is difficult to achieve based solely on observable, clinical signs. Necropsies of pigs that have died to the PRRS virus have no physical lesions that are indicative of PRRS, except for lesions due to pneumonia (Rossow, 1998). Thus, serological testing is perhaps the most reliable and convenient method to detect for PRRS virus infection. The

pathogenesis of the PRRS virus is not completely clear. Initiation of PRRS virus infection is thought to originate from macrophage phagocytosis in the respiratory tract. The virus can replicate inside these cells and create symptoms, including, but not limited to, pneumonia, rhinitis, vasculitis, lymphadenopathy, and encephalitis (Rossow, 1998).

Despite the major costs associated with PRRS, there is not yet an effective vaccine or medication with accepted widespread use. This is because there is a wide variety of genetically diverse strains of the PRRS virus. Also, previous studies have indicated that herds that have been vaccinated with a PRRS virus vaccine can be infected by a different strain of the virus. This suggests that due to the constant antigen shift of the PRRS virus, vaccination is ineffective against the PRRS virus (Kimman et al., 2009). In place of a PRRS vaccine, animal scientists have explored the use of genetic markers to select for increased disease resilience to PRRS. Boddicker et al. (2012) identified a major Quantitative Trait Loci (QTL) on *Sus scrofa* chromosome 4 (SSC4) that explained 15.7% and 11.2% of the genetic variance of viral load and weight gain in nursery pigs that underwent an experimental PRRS disease challenge. The QTL region of 0.5 Mb was found to be in high linkage disequilibrium (LD) and is tagged by the SNP WUR10000125 (WUR). In mammals, this region contains five Guanylate Binding Protein (GBP) genes, which have previously been observed to have strong effects on immune regulation and in mediating the inflammatory immune response in mice (Shenoy et al., 2012). Koltes et al. (2015) identified a splice site mutation in the GBP5 gene as the putative causative mutation for this QTL and found that that the WUR SNP was in complete LD with the GBP5 SNP. However, the latter conclusion was based on limited data from one genetic line. Literature on the effect of the SSC4 QTL is not limited to PRRS. Dunkelberger et al. (2017) observed that pigs co-infected

with both Porcine Circovirus Type 2b (PCV2b) and PRRS, but vaccinated for PRRS, had significantly lower viral load of PCV2b.

Porcine Circovirus 2: Another major infectious swine virus is Porcine Circovirus 2 (PCV2). This is a small, nonenveloped virus of 17 nm in diameter that is commonly found in swine farms. Serological studies indicate that PCV2 infection is found worldwide, especially in regions with dense swine production (Segalés et al., 2005). This virus is associated with several other disease symptoms, including post weaning multisystemic wasting syndrome (PMWS), systemic disease, reproductive failures, respiratory problems, enteric diseases, and nephropathy syndrome (PDNS), which are collectively known as porcine circovirus associated diseases (PCVAD) (Segalés et al., 2005). PMWS is most observable in newly weaned piglets and is characterized by wasting, paleness, and respiratory distress (Segalés et al., 1998). Weaned piglets that are afflicted with PMWS may have the same observable symptoms as PRRS (Harding, 2004). The most common symptom from PCV2 enteric disease (PCV2-ED) is constant diarrhea (Segalés et al., 2012). PCV2 reproductive disease (PCV2-RD) is characterized by abortions, mummifications, stillbirths, and reproductive failure at late gestation (Segalés et al., 2012). Last, PDNS is characterized by anorexia, depression, reluctance to move, and dark red papules on the pig's skin (Segalés et al., 2012). The most problematic, however, is arguably PMWS; Armstrong and Bishop (2004) estimated the cost of PMWS in the United Kingdom to be 35 million pounds per year.

PCV was first detected and isolated from the pig kidney cell line PK-15 in a study that suggested that PCV is nonpathogenic (Tischer et al., 1974). However, a later study indicated that large quantities of the PCV antigen were observed within the lesions of piglets affected with PMWS (Clark and Harding, 1997). This inconsistency of the nature of PCV led to nucleotide

sequencing of two PCV isolates (Hamel et al., 1998), which determined that there were two different types of PCV: PCV type 1 and PCV type 2, where the latter was strongly associated with disease like PWMS (Hamel et al., 1998). Later studies suggested that pigs experimentally infected with a PCV2 inoculum rarely showed symptoms commonly seen in commercial swine herds (Bolin et al., 2001). Later studies explored this further and discovered that clinical symptoms only occur in pigs coinfected with another infectious agent (Allan et al., 1999). These studies indicate that PCV2 alone rarely causes disease (Segalés et al., 2012).

PCV2 has a morbidity rate between 4 and 30% but this can increase to up to 60% in an epidemic. Mortality in affect farms ranges from 4 to 20% (Segales and Domingo, 2002). Diagnosis for PCV based on observational symptoms can be rather difficult because of the different clinical symptoms that PCV embodies. Also, due to the nature of PCV2, there must be a different diagnostic procedure for each of the PCVAD's, because the detection of antibodies or the presence of PCV2 does not identify the type of associated disease on the pig.

Several vaccines are available to combat some of the diseases associated with PCV2. Most farms rely on a PCV2 vaccine to control PWMS in young piglets. Many studies have been done to evaluate the use of PCV2 vaccination and have found favorable results. Young et al. (2011) showed that PCV2 vaccination can improve production parameters such as average daily gain and body condition scores.

Porcine Influenza Virus: Swine influenza virus (SIV), sometimes called Swine Flu, is a type A influenza virus from the Orthomyxoviridae family (Doyle and Gordon, 2008). Symptoms from SIV include fever, coughing, nasal discharge, and weakness over the course of one week. While SIV has a high morbidity rate (Gramer et al., 2006) and can cause severe disease, it has a low mortality rate, indicating that swine farms infected with SIV can slowly recover (Richt,

2006). SIV can infect pigs of all ages but symptoms are most often seen in younger pigs. The pathogenesis of porcine influenza viruses has been well studied and indicates that the primary target for this virus is the respiratory epithelium cells (Van Poucke et al., 2010).

All influenza viruses have major surface antigens comprised of hemagglutinin and neuraminidase glycoproteins (Gamblin and Skehel, 2010). The subtypes of influenza are based on the count of these glycoproteins. For example, classic SIV has one hemagglutinin and one neuraminidase glycoprotein, thus the nomenclature of classic SIV is HxNy; an example is pandemic H1N1. There are several different strains of swine influenza, including H2N2 and H1N2 (Crisci et al., 2013) that differ in the composition of these surface-level glycoproteins. These differences are created as a result of four main processes: antigen drift, antigenic shift, re-assortment of genes in a concurrent infection with a different pathogen, or cross-species transmission (Diaz et al., 2013).

Treatment for SIV is limited. Antiviral treatments are not licensed for use against SIV in commercial swine and, thus, vaccination is currently the main strategy for control of SIV. Unfortunately, there are many different strains of influenza virus and vaccination against one does not necessarily elicit protection against others (Vincent et al., 2008; Reeth et al., 2004). One strategy to mitigate the risk of SIV is relying on maternally derived antibodies by vaccinating breeding sows with an inactivated vaccine to stimulate passive antibody transfer to suckling pigs. This in turn reduces transmission of SIV between piglets (Romagosa et al., 2012). However, other studies have indicated that this strategy of using maternally derived antibodies has limited potential in preventing infection with swine influenza and may offer only partial protection (Cador et al., 2016).

Immune System

Swine in commercial barns face a myriad of pathogens in their lifetime. The best way to protect pigs from pathogens is by quarantining the pigs from any source of pathogens. This is done for pigs in the nucleus herd, which are kept in rigorously biosecure units to minimize possible transmission of any swine disease. This is, however, not feasible for commercial swine herds. Pigs in commercial facilities are often faced with a wide variety of infectious swine pathogens. The role of the immune system is to protect the animal from pathogens that they may face in their lifetimes. The following section will review the mammalian immune system, in particular the innate and active immune response, and conclude with a discussion on disease tolerance, resistance, and resilience.

The role of the immune system is to defend the body against harmful pathogens. There are two branches of the immune system (1) the innate immune system, and (2) the adaptive immune system. Innate immunity is sometimes called “non-specific immunity” while adaptive immunity is sometimes called “specific immunity”, as these two branches have different strategies for dealing with harmful pathogens. The innate immune system is very rapid and is the first line of defense against a pathogen. It is not selective, which means that it eliminates foreign invaders simply on recognizing whether the pathogen is of self or non-self. The adaptive immune system on the other hand, provides a slower response but results in a very specific response to a pathogen. The relationship between the innate and adaptive immune systems is that the immediate and broad innate response creates time for the slow, yet specific adaptive response to develop. When the adaptive immune response has been completed for a specific pathogen, the body has specialized mechanisms of ‘memory’ that allow an expedited response to that same

pathogen the next time it appears in the body. This section will discuss the main parts of the innate and adaptive immune systems.

Innate Immune System – Physical Barriers: The first external barrier to pathogens is the skin, which is not only the body's largest organ but also the boundary between our bodies and the environment. The skin passively acts as a physical barrier that prevents pathogens from entering the body. Recent studies have shown that the skin is home to trillions of microorganisms, including commensal communities of microbiota that maintain a fundamental level of surface level protection from harmful microbes (Naik et al., 2015). However, cuts and wounds leave an opening in the skin that allow pathogens to enter. If left untreated, open wounds allow harmful pathogens to enter and cause direct damage to the animal. To prevent this, physical insults resulting in wounds will cause the body to undergo what is called the "cascade effect" which is a repair mechanism consisting of a series of steps that allow the body to repair the wound.

While the skin is an effective physical barrier, many pathogens enter an animal's body through food and water. A physical barrier that can protect an animal from ingested pathogens is the acidic condition created by acids in the stomach, which assist in chemically denaturing the majority of pathogens that find their way into an animal's stomach. Some pathogens can enter the body through the air and make their way through nasal passages, trachea, and the urinary, urethral, and vaginal tracts. These routes, however, are lined with mucous, a type of sticky tissue that acts as another physical barrier. These sticky linings are produced by goblet cells that reside in mucosal membranes, and are particularly effective in trapping airborne pathogens in the nasal and trachea. In these passages, small tiny hairs called cilia can move mucous outside of the body in a wave like motion, and with it, the trapped pathogens. An enzyme called lysozyme is also

produced as part of the mucous, which helps destroy pathogens that are caught in the sticky lining.

Innate Immune System – Cellular Components: When the three physical barriers are unable to keep a pathogen outside of the body, the organism's cellular innate immune response will implement a series of steps to prevent replication and spread of the pathogen inside the body.

The first step after an infection is the inflammatory response. The infection may cause tissue damage which causes mast cells to release a compound caused histamine. Histamine can then create two separate reactions, (1) vasodilation of local blood vessels and (2) increased local capillary permeability. Both of these reactions cause similar cardinal signs, such as redness, pain, swelling, and heat. The rate of blood flow to different parts of the body depends on the diameter of the blood vessels. Thus, dilation of the vessels by histamine reduces resistance and, thus, more blood flows into the area of infection. The greater blood flow also increases the delivery of healing factors, such as plasma proteins or clotting factors, which is important for infections caused by an opening in the skin. Histamine will also cause an increase in capillary permeability, which means that the pore size between the endothelial cells that form the walls of the capillary is increased. As a result, more fluid can accumulate in the area, which also includes the influx of macrophages. Macrophages and neutrophils are two types of leukocytes that the body implements heavily in the innate immune system. Neutrophils are the most abundant type of white blood cell in the body and are known for their mobility. They can easily move between vessels and tissues, quickly congregating in areas of the body that other cells cannot. Neutrophils are some of the first cells to arrive in an area of inflammation and will start the phagocytosis of any foreign invader, which is a nonspecific strategy for dealing with an infection. Similar to

neutrophils, macrophages will also implement phagocytosis of pathogens, but due to their large size, they often require histamines to expedite their travel to the inflammation site. Macrophages are also involved in cleaning up cell debris, such as dead neutrophils, which is important for resolving the inflammation.

Another type of cell that is specific to the innate immune response is the natural killer (NK) cell. The NK cells float freely in the body and are consistently on patrol for problematic cells. They are in charge of destroying cells infected or cancerous cells, based on an altered major histocompatibility complex (MHC), which are glycoproteins that are prominently displayed on surface of all cells. Hence, the name "natural killer" as a "non-natural" MHC of a cell automatically indicates to the NK cells that there is a problem with this cell, requiring no other activation for the NK cell to destroy the problematic cell. While NK cells are known for their use in the innate immune system, there is some recent evidence suggesting that NK cells also have a role in the adaptive immune response, and can develop antigen specific memory, a function of the adaptive immune response (Vivier et al., 2011).

Another part of the innate immune response is the interferons. Interferons are a large group of cytokines, which are chemical messengers the cell uses to communicate with other cells. While there are many documented roles of interferons, generally, they are chemical messengers that alert nearby cells of a viral infection. In other words, interferons will interfere with viruses. Take for example, a healthy cell that has now been infected with a virus. The virus requires another cell to survive, by taking over its machinery and replicating the virus DNA inside the host cell. While this happens, the infected cell will release interferons into the extracellular fluid, outside of the cell. These interferons travel and encounter surface receptors of other healthy cells, alerting them that a nearby cell has been infected with a virus. This causes

these alerted cells to heighten their viral defenses by creating a wide variety of inactivated enzymes. When a virus does infect those cells, the interferon induced enzymes will activate like a trap, effectively breaking down viral messenger RNA while reducing or even inhibiting viral protein synthesis. This means the virus can no longer replicate inside the cell.

Adaptive Immune System - Cell Mediated Immune Response: The common mechanism of the innate immune system involves the removal of pathogens based on identification of self or non-self. While this method is fast, it is not efficient, as future infections of the same pathogen will likely require the same inflammatory response. The innate response does, however, provide time for the body to develop immunological memory against that specific pathogen. This is called the adaptive immune response and relies on use of antigens. An antigen can be defined as a unique molecule from the cell surface of a pathogen. It generally triggers an immune response in the body, which creates antibodies against it. The adaptive immune response can be separated into two responses: (1) cell mediated and (2) humoral.

The cell mediated immune response involves the cytotoxic T cell, which has the ability to release proteins called perforins when in the neighborhood of an infected cell, which increases the permeability of the infected cell, initiating it for apoptosis. This will inevitably destroy the cell. For viruses in particular, this means that the virus cannot replicate inside the cell, a requirement for virus survival. This behavior from the cytotoxic T cell is stimulated when it encounters an infected cell that has presented an antigen from the infectious agent, a process known as antigen presentation.

Another type of T cell that is important for the adaptive immune response is the helper T cell. T helper cells often require the help from antigen presenting cells, such as macrophages which can phagocytize infected cells or pathogens without specificity, as previously discussed.

Macrophages can process the pathogen and present the pathogen's antigen on its own surface, which can be presented to a T helper cells that has a matching receptor for the antigen. This leads into the humoral immune response.

Adaptive Immune System - Humoral Immune Response: The humoral response involves the development of pathogen specific antibodies. When the T helper cell is activated by a matching antigen that is presented by a cell, it will wait until a B cell is also activated by the same antigen-T helper cell complex. When both the B and T cells are activated by the same antigen, this will cause the release of cytokines that induce that particular B cell to divide rapidly. The resulting cells are either plasma cells that release antibodies, or identical copies of the B cell, which are called memory B cells. This rapid division eventually causes the production of a large number of antibodies that are specific to that particular antigen, that are released into the bloodstream. Antibodies are Y shaped proteins that attach themselves to the binding site of any corresponding antigen found in the bloodstream. Since pathogens carry these antigens on the surface of the cell, antibodies will ultimately cover the surface of the pathogen by binding to the antigens. This binding can result in a myriad of different effects that impede the pathogen's ability to cause damage. Antibodies can activate macrophages to instantly destroy pathogens that are covered in antibodies, or they can neutralize important parts of a pathogen that are necessary for it to replicate. Overall, the main goal of the adaptive immune response is to create antibodies that are specific to a pathogen's antigen, which means the body will now be able to efficiently handle the same pathogen the next time it appears in the body.

Disease resistance, resilience, and tolerance

Animal breeders have a great interest in enhancing the overall immune responsiveness of the host animal. Disease occurs when environmental factors, such as the level of disease in a

swine barn, overwhelm a host animal's genetic disposition to combat disease. One term commonly used is disease resistance, which can be defined as the animal's ability to exert control over the pathogen's life cycle (Bishop et al., 2012). Animals with high disease resistance can maintain productivity by limiting the replication of the pathogen. Therefore, animals that are highly resistant to a pathogen would have a lower pathogen burden, or amount of pathogen in the body. Pathogen burden can be quantified by using assays, such as Taqman PCR assays, which is what was used in the PRRS Host Genetics Consoritum (PHGC) trials to measure PRRS viral load in pigs (Boddicker at al., 2012). Disease tolerance, on the other hand, can be defined as the animal's ability to minimize expression of disease on performance under an environment with a given level of pathogen burden (Bishop and Stear, 2003). One main challenge associated with measuring an individual animal's resistance and tolerance is that pathogen burden within each animal needs to be measured at several key time points using precise assays. Disease resilience is a combination of disease tolerance and disease resistance and can be defined as the productivity of an animal during the course of an infection (Albers et al., 1987; Doeschl-Wilson et al., 2012; Mulder and Rashidi, 2017). Thus, resilient animals that are infected with disease are able to continue their productivity at a rate similar to non-infected animals.

Tools to Evaluate Disease

The mammalian immune system is complex. It consists of a network of organs that orchestrates a wide variety of cell-to-cell interactions and biological pathways in response to a pathogen. To investigate the biological manifestations of disease in an organism, one strategy is to analyze separate constituents of the immune system using an assay. Assays are laboratory tools that can be used to quantify the presence of a particular biomolecule, including the presence

of a pathogen. The following technologies are used to measure different components of an animal's response to disease, with some of these used in the studies described in Chapters 3 and 4.

Cell Flow Cytometry: Cell flow cytometry is a widely used tool to sort and measure a sample of cells. This machine relies on the Coulter Counter, which was developed in the 1950s by Wallace H. Coulter and is used in almost every automated cell counter to this day. The basic objective of cell flow cytometry is to measure the physical properties of a cell as they flow through a microchannel. Named after Coulter himself, a Coulter Counter can measure the change in electrical impedance when a particle, such as a cell, is passed through a microchannel. Electrical impedance changes when the cell enters and leaves the channel, providing a reliable system to count cells. This technology was first successfully implemented to determine the composition of blood (Don, 2003). Today, modern cell flow cytometers can count thousands of cells all at once and can even be configured to sort cells with the addition of the dimension of optical physics to the Coulter Counter. This augmentation relies on the use of a laser to excite cells that pass through the channel. Each cell that passes through the laser will emit a special wavelength of light, which can be measured using a specialized sensor. Software can analyze these patterns, allowing information such as the size, surface characteristics, and immunophenotyping to be gathered. One study specific to swine has used cell flow cytometry to quantify T cells and to observe the rearrangement of immunoglobulin heavy chain genes (Sinkorava et al., 2019).

Quantifying the presence of a pathogen: There are many types of assays to identify the presence of a pathogen in a sample, two of which were used in the studies included this thesis. The first is the polymerase chain reaction (PCR) assay for the identification of pathogens. A PCR

assay allows the exponential amplification of a gene or a DNA fragment. These amplified products can then be separated on a gel using electrophoresis. This then allows for the identification of the pathogen. The second is a Fluorescent Assay, which uses a light source such as UV radiation to excite molecules in a sample. Initially, these molecules are at a ground state, but when hit with UV light, they will absorb light, which will be emitted when they return to a ground state. The wavelength and intensity of this emitted fluorescence can be detected and measured. The output of a fluorescent assay often includes vivid displays of color to visualize the location of targeted compounds, such as a pathogen. Johani et al. (2011) used fluorescently-labeled antibodies to detect influenza virus in pigs. A third assay commonly used is the enzyme-linked immunosorbent assay (ELISA). This assay can determine the presence of ligands, such as an antigen, in a sample. Antibodies that are linked to an enzyme, are mixed with a sample containing antigens. The critical step is that there must be a strong affinity between the antibody and the antigen, thus the reagents used are very important for success. Then, when the substrate for the enzyme is provided, an enzymatic reaction will occur, leading to a visual change in color. The presence of color indicates that the corresponding antigen to the provided antibody is present in the sample. Gamble et al. (1983) used ELISA to diagnose pigs with swine trichinosis.

Mitogens

Mitogens are chemical stimulants that are known to activate the proliferation of peripheral blood mononuclear cells and are commonly used in immunological experiments. Many mitogens are lectins, which are proteins that are derived from plants or bacteria (Mak and Saunders, 2006). Mitogens have a similar effect as antigens, can bind with receptors like antigens, resemble pathogen aggression, and trigger lymphocyte mitosis immediately after

recognition (Ko et al., 1979). Since mitogens can induce lymphocyte proliferation, they can be used to assess the responsiveness of an animal's immune system to a potential pathogen. In two papers that previously utilized mitogen stimulation to measure immunity traits in swine, both the Edfors-Lilja (1994) and Flori (2011) group were able to measure lymphocyte by measuring DNA synthesis through the use of [3H]-thymidine during mitotic cell division. A scintillation beta-counter was then used to measure the radioactivity of [3H]-thymidine incorporated into the DNA of the newly formed immune cells, providing both groups a direct measurement of mitotic cell division. In the following, the five mitogens that were used in the study described in Chapter 4 will be described: Concanavalin A (Con A), Phytohemagglutinin (PHA), Poke Weed Mitogen (PWM), Lipopolysaccharide (LPS), and Phorbol myristate acetate (PMA).

Concanavalin A: Concanavalin A (Con A) is a tetrameric protein that is extracted from jack beans (Canavalia ensiformis). Also considered to be the most useful plant lectin, Con A has been shown to function as a T cell inducer. Con A works as a mitogen by binding to glycoproteins in the blood, particularly the T cell receptor (Kay 1991). One of the first mitogens to evaluate swine immunology was Con A, in a study in a study in which Con A induced immune cell proliferation was found to be correlated with other immune traits (Edfors-Lilja et al., 1994). Another study used Con A to study the blastogenic response of swine lymphocytes in blood and observed that Con A was successful in stimulating the peripheral blood mononuclear cells in pig blood, including T cells (Lin (2012). These studies suggest that Con A can be used to stimulate the pig's blood in vitro to observe immune proliferation.

Phytohemagglutinin: Phytohemagglutinin (PHA) is an extract from the kidney bean plant (Phaseolus vulgaris). PHA has been found to induce the proliferation of T cells (Mire-Sluis et al., 1987). Lamers (1999) used human blood to show that PHA can induce T lymphocyte

expansion. However, PHA is not limited to just T cell proliferation. Piguet et al. (1972) observed that PHA can induce B cell proliferation as well. These studies suggest that PHA can be used to study both T and B cell proliferation in swine.

Poke Weed Mitogen: Poke Weed Mitogen (PWM) is another plant lectin derived from the pokeweed plant (Phytolacca americana). PWM is commonly used as a stimulus to induce B cell differentiation in humans and rats (Blomgren et al., 2009 and Jørgensen et al., 1972). Paul et al. (1979) used pokeweed to stimulate blood lymphocytes in swine. Using immunofluorescence, they were able to detect the replication of both T and B cells. Another study found that PWM induces a wide variety of T cell subsets such as CD4 +, CD8 +, and gamma delta T cells (Dorn et al., 2002). These studies indicate that PWM is an effective mitogen for the stimulation of both B and T cells in swine.

Lipopolysaccharide: Lipopolysaccharide (LPS) is an endotoxin and is a major component of the outer membrane of gram-negative bacteria. It is important for structural integrity and the stabilization of the bacterial membrane (Rietschel et al., 1994). Since its discovery in the early 1900s, many studies have performed on the effect of LPS on the mammalian immune system. Schmitt et al. (2004) discovered that the body recognizes LPS due to a protein found on the surface of cells, which are named toll-like receptor 4 (TLR4). Once identified, the immune system will often react vigorously and immediately to an endotoxin such as LPS. Tough, et al. (1997) used LPS as a mitogen in mice and indicated that LPS can cause polyclonal activation of B cells and T cells. LPS was one of the mitogens used in the earlier studies of mitogen induced immune cell proliferation in swine (Flori et al., 2011).

Phorbol Myristate Acetate: Phorbol Myristate Acetate (PMA) is extracted from croton oil, obtained from the seeds of the croton tree (Croton tiglium). Similar to phytohemagglutinin in

effect, studies have shown that PMA can act as a mitogen as it stimulates T cell proliferation in humans (Touraine et al., 1977). One early study observed that PMA induces the proliferation of lymphocytes and affects glutamine metabolism in swine (Wu, 1996). Stepanova et al. (2012) showed that stimulation of whole blood cells from swine with PMA resulted in the proliferation of CD3+ and CD4+ T cells.

Previous Mitogen Assay Studies in Pigs

The use of mitogens to evaluate of immune cell proliferation in swine has been performed before. One of the earliest studies estimated the genetic parameters of porcine immune traits, including Con A induced immune cell proliferation (Edfors-Lilja et al., 1994). Using blood extracted from 124 Yorkshire pigs of 8 weeks, the heritability estimates of Con A induced immune cell proliferation in-vitro was determined to be 0.38 ±0.21 at 48 hours after stimulation with Con A at a concentration of 10 μg/ml. This study also found positive phenotypic correlations of this trait with both interleukin-2 and IFN-alpha production in separate samples at 48 hours. Genetic correlations of immune traits with the Con A assay phenotypes were not determined. Another study used Con A, PMA, and LPS to estimate the genetic parameters of 32 immune traits, including mitogen-induced lymphocyte proliferation in-vitro, using blood samples collected on 443 Yorkshire pigs at eight weeks of age, three weeks after vaccination against *Mycoplasma hyopneumoniae* (Flori et al., 2011). This study found moderate estimates of heritability for the proliferation of immune cells at 48 hours post stimulation using Con A (0.36 ±0.20), PMA (0.27 ±0.20), and LPS (0.31 ±0.19). However, these estimates were not significantly different from zero. The estimate of heritability of Con A was, however, consistent with the estimate of Edfors-Lilja et al. (1994), although both studies suffered from relatively large standard errors of heritability estimates. Flori et al. (2011) also reported very

small genetic correlations among these phenotypes and other immune related traits, with large standard errors.

Gao et al. (2010) used DNA microarrays in a transcriptome analysis to observe the proliferation of porcine PBMCs when stimulated with LPS and a mixture of PMA and ionomycin for 24 hours. This study showed that stimulation with these two mitogens yielded distinct immune responses, which was verified by the activation of different sets of genes. However, the genes that were activated after stimulation with either mitogen shared the top biological functions in catalogs from Diseases and Disorders, Molecular and Cellular Functions, and Physiological System Development and Function, which suggested that the effects of the two stimulants were broadly similar (Gao et al., 2010). However, it was found that the relative representation of each biological function differed between the two mitogens. For LPS, the most represented genes were related to Disease and Disorder, while for PMA and ionomycin, the most represented genes were related to Molecular and Cellular functions.

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CHAPTER 3. EFFECT OF GENOTYPE AT A GENETIC MARKER FOR THE *GBP5* GENE ON RESILIENCE TO A POLYMICROBIAL NATURAL DISEASE CHALLENGE IN PIGS

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Abstract

A genomic region on chromosome 4 that is tagged by single nucleotide polymorphism (SNP) WUR0000125 (WUR) was previously found to be associated with host response to porcine reproductive and respiratory syndrome (PRRS) virus infection. The objectives of this study were to 1) determine whether genotype at the WUR SNP is also associated with resilience to a natural polymicrobial disease challenge, 2) investigate the relationship of genotype at the WUR SNP with genotype at its putative causative mutation in the *GBP5* gene, and 3) compare the

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association of the WUR and *GBP5* SNPs with host response to PRRS virus (PRRSV) infection. Data from two studies were used: 1) Eight trials of the PRRS Host Genetic Consortium (PHGC), in which ~200 naïve crossbred nursery pigs/trial were infected with the NVSL-97-7895 strain of PRRSV to study the effects of genotype at the *GBP5* and WUR SNPs on viral load and weight gain post-infection; 2) a natural disease challenge, where 3139 naïve crossbred nursery barrows were entered into a grow-finish facility that was seeded with multiple pathogens to maximize expression of disease resilience. Results from the PHGC trials showed that the WUR and GBP5 SNPs are in high but not complete linkage disequilibrium (r^2=0.94). A haplotype analysis showed that discordant genotypes between the WUR and *GBP5* SNPs were due to genetic recombination and not the result of genotyping errors. We were unable to determine whether GBP5 or WUR had a stronger effect on phenotype. Results from the natural disease challenge indicated that the favorable allele for the WUR SNP was significantly associated with greater average daily gain (p=0.02) and lower numbers of treatments in the challenge nursery (p=0.05) and across the nursery and finisher (p=0.01). Therefore, swine breeders can continue to use the WUR SNP not only as a marker for resilience to PRRSV infection, but also as a marker for disease resilience to a polymicrobial disease challenge.

Introduction

Infectious disease has large economic costs in the swine industry and affects animal welfare. One such disease is Porcine Reproductive and Respiratory Syndrome (PRRS), caused by the PRRS virus (Holtkamp et al., 2013). The PRRS virus (PRRSV) affects both young pigs and pregnant sows. Infected young pigs show respiratory symptoms such as pneumonia, fever, and lethargy, while infected pregnant sows can have abortions or give birth to dead or

mummified piglets (Holtkamp et al., 2010). The total cost to the United States swine industry was estimated to be around 664 million US dollars per year (Holtkamp et al., 2013). The PRRSV can mutate rapidly, allowing a great variety of PRRSV strains to exist, which reduces the effectiveness of preventative vaccines and medications (Fang et al., 2007).

One strategy to mitigate costs due to PRRS and other infectious porcine diseases is to genetically select animals with higher disease resilience, which is defined as the ability to maintain relatively undiminished performance levels under infection (Albers et al., 1987; Doeschl-Wilson et al., 2012; Mulder and Rashidi, 2017). However, direct selection of disease resilience is difficult because the elite nucleus populations that genetic selection is practiced in must be kept under biosecure conditions. An alternative approach is to select on genetic markers that are predictive of disease resilience, as this can be practiced in high-health nucleus herds.

Previous research has shown that the *Sus scrofa* chromosome (SSC) 4 contains a Quantitative Trait Locus (QTL) for host response to PRRSV infection (Boddicker et al., 2012). Single Nucleotide Polymorphisms (SNPs) in this QTL region of 0.5 Mb were in high linkage disequilibrium (LD) in the breeds and crosses investigated by Boddicker et al. (2012, 2014) and the region was tagged by the single nucleotide polymorphism (SNP) WUR10000125 (WUR). In pigs, this QTL region contains five *Guanylate Binding Protein* (*GBP*) genes, which are recognized for their effects on immune regulation and for mediating inflammatory immune response in the mouse (Shenoy et al., 2012). Koltes et al. (2015) identified a splice site mutation in the *GBP5* gene as the putative causative mutation for this QTL and showed that the WUR SNP is in complete LD with the *GBP5* SNP. However, the LD result was based on limited data from one genetic line. Literature on the effect of this QTL are not limited to PRRS. Dunkelberger et al. (2017) showed that the WUR SNP is also associated with porcine circovirus 2b (PCV2b)

viral load following co-infection with PRRSV and PCV2b of pigs that were vaccinated for PRRS, although Kreikemeier et al. (2015) did not identify the SSC4 QTL region to be associated with PCV2b viremia in pigs that were only infected with PCV2b. In commercial production, pigs are typically exposed to multiple pathogens and, thus, it is also important to evaluate the effect of the SSC4 QTL on disease resilience under a polymicrobial disease challenge.

To further investigate the effects of the WUR SNP and the putative causative mutation in the *GBP5* gene, three objectives were pursued here: 1) determine whether the SSC4 QTL (the WUR SNP) is associated with resilience to a polymicrobial disease challenge in pigs, 2) investigate the LD of the WUR SNP with its putative causative mutation in the *GBP5* gene in different genetic lines, and 3) compare the association of the WUR SNP versus that of the putative causative mutation in the *GBP5* gene with host response to a PRRSV infection.

Materials and Methods

Porcine Reproductive and Respiratory Virus (PRRSV) Infection Trials

Animals and data

To investigate the relationship between and effects of genotype at the WUR SNP and the putative causative mutation in the *GBP5* gene, data from the first eight trials of the PRRS Host Genetics Consortium (PHGC) (Lunney et al., 2011) were used. The animal experiments for the PHGC were approved by the Institutional Animal Care and Use Committee of Kansas State University. Detailed accounts of the design and data collection in the PHGC trials are in Lunney et al. (2011) and Boddicker et al. (2012, 2014). Briefly, data were available from eight batches of about 200 high-health commercial crossbred pigs, which were transported after weaning to Kansas State University, where each batch was subjected to an experimental PRRSV challenge.

Pigs from the first three batches came from the same breeding company but each batch after that came from a different company (Boddicker et al., 2014). After a one-week acclimation, all pigs were experimentally infected (intranasal and intramuscular) with the NVSL-97-7895 strain of the PRRSV. Blood samples were collected at -6, 0, 4, 7, 14, 21, 28, 35, and 42 days post-infection (dpi) and all pigs were euthanized at 42 dpi. Viremia was measured using a TaqMan PCR assay for PRRSV RNA. Viral load was calculated for pigs that had viremia records on day 0 and at least 5 other days, as the area under the curve of log2-transformed viremia at 0, 6, 7, 11, 14, and 21 dpi. Body weights were collected at 0, 7, 14, 21, 28, 35, and 42 dpi. Weight gain was calculated as body weight at day 42 minus day 0, except for pigs that died before 42 dpi, as detailed by Boddicker et al. (2012). All pigs were genotyped with Illumina's Porcine SNP60 BeadChip (San Diego, CA), which includes the WUR SNP but not the *GBP5* SNP. Thus, all pigs were also genotyped separately for the *GBP5* SNP, using a custom Sequenom panel (Geneseek Inc.).

Statistical Analyses

Linkage disequilibrium between the WUR and *GBP5* SNPs was calculated as the correlation between the genotypes at the WUR and *GBP5* SNPs, both within and across the eight trials. For WUR, the three genotypes were AA, AG, and GG, where G is the favorable allele. For GBP5, the three genotypes were GG, GT, and TT, where T is the favorable allele. With complete LD, the genotype at WUR should match the genotype at GBP5 exactly.

In order to determine if discordant genotypes between these two markers were due to genotyping errors or genetic recombination, the Haploview software (Barrett et al., 2005) was used to identify the haplotypes that were present within the 1 Mb region across the trials and the frequency of each haplotype. In order to determine discordant genotypes were the result of

genotyping errors, a preliminary analysis was done to see if changing the allele at either marker matched another haplotype with a reasonable frequency. In order to determine if a discordant haplotype was due to a single recombination event between *GBP5* and WUR, each discordant haplotype was investigated to see if recombining two non-discordant haplotypes would yield the discordant haplotype in question. Finally, pedigree information (sire and dam) was used to determine whether pigs with the same discordant haplotype within a genetic source had a common parent. If the pigs that carry a discordant haplotype within a trial shared a parent, this indicates that the parent carried that haplotype, and therefore, the discordant genotype at the two markers are not due to genotyping error, but due to genetic recombination. If there was not a common parent, the genomic relationship (computed in Hess et al., 2018) between pairs of discordant pigs was evaluated.

The effects of genotype at the WUR and *GBP5* SNPs on PRRSV viral load and weight gain were evaluated by fitting them separately as fixed effects in a linear mixed model with additional fixed effects of trial, parity of the sow within trial, and sex, the covariates of initial age and weight, and the random effects of pen within trial, litter, and animal genetic effects. To determine which SNP had a stronger association with phenotype, both SNPs were fitted simultaneously in the model and significance of adding the *GBP5* (WUR) SNP when the WUR (*GBP5*) SNP is already in the model was evaluated in ASReml 4.0 (Gilmour et al., 2009). Significance was declared at $P \leq 0.05$, with suggestive significance at $p<0.10$.

Natural Disease Challenge Model

Animals and protocols

Data from the natural disease challenge described by Putz et al. (2019) were used to investigate the effect of the WUR SNP on response to a severe polymicrobial challenge. The natural disease challenge enabled the expression of disease resilience related phenotypes, facilitating the recording of these traits. The natural disease challenge was carried out in accordance with the Canadian Council on Animal Care guidelines (CCAC; https://www.ccac.ca/en/certification/about-certification) and was approved by the Protection Committee of the Centre de Recherche en Sciences Animales de Deschambault (CRSAD) and the Animal Care and Use Committee at the University of Alberta (AUP00002227). The project was conducted and overseen by the Centre de développement du porc du Québec (CDPQ) and the herd veterinarian, along with project veterinarians. The CDPQ research facility was equipped with tools and staff that allowed for detailed phenotype recording, blood sampling, and strict biosecurity. Collection of the data used started in late 2015 and concluded in early 2019.

A detailed overview of the design, data collection, and genotyping for the natural disease challenge is in Putz et al. (2019). Briefly, the CDPQ facility was segmented into three physical compartments: (1) a separate quarantine nursery, where the crossbred (Landrace and Yorkshire F1 barrow) piglets were kept for around 19 days after weaning from a high-health multiplier and transported to the facility; (2) a challenge nursery, where the naïve piglets were subjected to the disease challenge for around 28 days; (3) a finishing unit, where the pigs were raised to slaughter weight for 69 days on average. Every three weeks, a new batch of 60 or 75 naïve piglets was introduced in the quarantine nursery, alternating between the seven member genetics companies

of the PigGen Canada consortium. For the current study, data from the first 50 batches were used.

The polymicrobial challenge was established using seeder pigs, which were naturally infected pigs that were obtained from farms in the area, and were introduced together with the first four batches of naïve pigs to replicate diseases that occur in commercial barns. After inoculation of the challenge nursery, each new batch of naïve piglets was allowed fence-line contact with the previous batch for ~1 week to maintain the disease challenge, circumventing the need for labor-intensive re-inoculation of the challenge nursery. To maintain controlled disease pressure and acceptable levels of mortality that were instituted by the Animal Protection Committee, licensed veterinarians closely monitored the health of every batch through established veterinary protocols, including removal of fence-line contact to infect the new batch of pigs when the disease pressure became too high. After the first seven batches vaccination for PCV2 was added to the quarantine protocol. Pigs were treated from a list of 10 antibiotics, as needed, depending on their individually expressed clinical symptoms. Preventative mass treatments were also given through the drinking water, usually by batch. Decisions to euthanize pigs were by farm staff, based on defined endponts, with oversight from licensed veterinarians.

The following viruses were identified in the challenge nursery during the study: three strains of PRRSV, two strains of Swine Influenza Virus, PCV2, and Porcine Rotavirus A. Bacterial pathogens identified included *Actinobacillus pleuropneumoniae, M. hyo, Streptoccus suis, Haemophilus parasuis, Brachyspira hampsonii, Salmonella sp., Cystoisopora suis, Erysipelothrix rhusiopathiae,* and *Staphylococcus hyicus*. This list of bacterial pathogens is not comprehensive and several other minor bacterial pathogens that were not identified may have

been present. In addition, not all bacterial pathogens were found in every batch, reflecting the heterogeneity and dynamics of disease pressure in commercial farms.

Data Collection

The phenotypic data that were collected on all pigs in the natural disease challenge were described in detail in Putz et al. (2019). In addition to identification of the pig's dam, and the quarantine, nursery, and finisher pen it was in, information collected on each pig included date of birth, age at weaning, and whether or not they received pen enrichment (non-edible toys) in the quarantine and challenge nursery (used for half of the pens of batches 41 to 50), as part of a separate study to evaluate the impact of environmental enrichment on resilience. Individual body weights were recorded, starting with entry into the quarantine nursery, at entry and at exit of the challenge nursery, and every three weeks thereafter in the finisher. Pigs that were severely diseased were weighed more often. For the calculation of average daily gain (ADG), LOESS predicted daily weights were used, as described by Putz et al. (2019). Groups of pigs were sent to slaughter every three weeks. Pigs from a batch that had not met the targeted market weight of 135 kg were delayed from slaughter for three weeks. Carcass traits that were recorded at the slaughter house included back fat, loin depth, and lean yield.

Feed was available *ad libitum* throughout the two nursery and finisher units. Individual feed intake was measured and quantified in the finisher, as described by Putz et al. (2019), resulting in a daily total for each animal for the amount of feed consumed (kg) and time spent in the feeder (duration in minutes). Feed intake traits evaluated included Average Daily Feed Intake (ADFI), Feed Conversion Ratio (FCR), and Residual Feed Intake (RFI), as described by Putz et al. (2019). Only pigs that survived to slaughter were analyzed for these traits. Several novel disease resilience phenotypes were derived from the daily feed intake data by Putz et al.

(2019), including the proportion of off-feed days and day-to-day variation in feed intake. The proportion of off-feed days was based on the proportion of days with a negative residual from 5% quantile regression of feed intake or duration on age across pigs, as described by Putz et al. (2019). Pigs with a higher proportion of off-feed days were considered less resilient than those with a smaller proportion of off-feed days. Day-to-day variation in feed intake was based on the root mean square error for daily feed intake or duration of ordinary least squares regression of feed intake or duration on age by pig, as described by Putz et al. (2019). Similar to proportion of off-feed days, pigs with a higher day-to-day variation in feed intake were expected to be less resilient than those with a smaller day-to-day variation in feed intake.

Treatments and mortalities were recorded by CDPQ staff. The number of treatments was a count of the total number of individual treatments that a pig received, which included any drug injection, but excluded group and batch level treatments. Clinical symptoms that were recorded included abscesses, arthritis, ataxia, black scours, conjunctivitis, diamond skin disease, digestive or respiratory problems, fever, greasy pig disease, grey or brown diarrhea, inappetence, lameness, problems with locomotion, convulsions, nasal discharge, cyanosis, rectal prolapse, dysentery, thumping, mites, sneezing, tail/ear/flank biting, and salmonella. To account for the smaller number of days at risk for pigs that died prematurely, the number of treatments was scaled to a standard length of 180 days for number of treatments to average age at slaughter, to 27 days for number of treatments in the challenge nursery, and to 100 days for number of treatments in the finisher. For pigs to have a record of treatments scaled to 180, they were required to reach 65 days of age. The number of treatments for respiratory symptoms was also analyzed in order to focus on treatments for PRRS. Mortality was also measured as a phenotype, identifying pigs that died in the challenge nursery, the finisher, or across both phases.

All pigs were genotyped using a commercial 650K SNP panel (Affymetrix), consisting of 658,692 SNPs, including the WUR SNP, but not the *GBP5* SNP. Details on the genotyping, filtering, and imputation of missing genotypes are in Putz et al. (2019).

Statistical Analyses

Data from the 3,126 pigs that were entered into the natural challenge facility in 50 batches were analyzed using a univariate linear mixed model that included the fixed effects of batch, entry age (covariate), and WUR genotype, and pen, sow, and animal genetics as random effects. Previous studies have shown that the G allele at the WUR SNP is rare in most commercial lines and that the G allele appears to be completely dominant over the A allele in its effects on host response to PRRSV infection (Boddicker et al., 2014). Therefore, pigs with at least one G allele were grouped and contrasts were calculated between genotypes AA and [AG+GG]. Significance was declared at $P \leq 0.05$, with suggestive significance at $P<0.10$. To incorporate animal genetic effects, a genomic relationship matrix was constructed from the SNP genotypes, with relationships between pigs from different companies set to 0. Mortality in the challenge nursery, the finisher pen, and across the nursery and finisher were also analyzed as continuous traits. For carcass traits, pre-slaughter weight, slaughter age, and slaughter date were added as covariates. All analyses were conducted using the lme4qtl package in R (Ziyatdinov et al, 2018).

Results and Discussion

Linkage disequilibrium between WUR and GBP5

Koltes et al. (2015) determined that the WUR SNP was in complete LD with the putative causative mutation in the *GBP5* gene ($r_2 = 1$) based on genotypes of 58 boars from a commercial

Yorkshire line of pigs, to such that the WUR SNP is an effective marker to select for improved host response to PRRS. Our more comprehensive analysis, using genotype data of 1387 pigs from PHGC trials 1 to 8, however, found the LD between these two markers to be incomplete, with $r^2 = 0.94$. The LD between the two SNPs ranged from 0.72 to 0.99 for pigs from the individual companies that contributed to the PHGC trials. **Table 3.1** displays the counts of genotypes of all 1387 pigs from the 8 PHGC trials, while genotype counts for each trial are in **Supplementary Table 3.1**. In total 43 of the 1387 animals had discordant genotypes (off-diagonals in **Table 3.1**), for which the genotype at the WUR SNP did not match the genotype at the *GBP5* SNP. The large majority of these were AG at WUR and GG at *GBP5;* however, all pigs with the GG genotype at WUR matched the TT genotype at *GBP5*.

In order to determine whether the mismatches between the genotype at the WUR and *GBP5* SNPs were due to genotyping errors or to recombination events, a haplotype analysis was conducted. Across all trials, 135 unique haplotypes were observed for the 1 Mb region around the WUR and *GBP5* SNPs, consisting of 47 SNP's. This window was narrowed down to 13 SNPs, with the WUR and *GBP5* SNPs at the 5^{th} and 9^{th} position respectively, yielding 18 unique haplotypes across the 13 SNP region. Among these 18 haplotypes, three had discordant alleles at the *GBP5* and WUR SNPs. Each of these 3 haplotypes could be generated through a single recombination event between the *GBP5* and WUR SNPs for two concordant haplotypes. Based on pedigree analysis, every pig that had a discordant genotype within a trial, shared at least one parent with another pig from that trial with the same discordant haplotype, except for one pair of pigs. The genomic relationship between these two pigs was, however, 0.45, suggesting that they shared a recent common ancestor that likely carried the discordant haplotype. Overall, the haplotype analysis provides substantial evidence that the discrepant genotypes were due to

recombination events, rather than genotyping errors. The recombination frequency between these the WUR and *GBP5* SNPs could, however, not be determined since the number of meiosis events was not available.

Effects of WUR and *GBP5* on host response to PRRSV infection

Results in **Table 3.2** show that estimates of the effects of the WUR and *GBP5* SNPs on weight gain and viral load following PRRSV infection were similar but not identical, because of the incomplete LD. Because the *GBP5* SNP is the putative causative mutation, it was expected to exhibit a stronger association with host response than the WUR SNP. However, when fitting both SNPs simultaneously, the p-value for the extra variation explained by the *GBP5* SNP, after the WUR SNP was already included, was not significant (p=0.46 for VL and p=0.23 for WG), suggesting that there is no statistical evidence that the *GBP5* SNP was more strongly associated with host response to PRRS than the WUR SNP. Because differences between the effects of the two SNPs were small, if not zero, the WUR SNP, which is present on most commercial SNP panels, can continue to be used to select for improved host response to PRRS. However, the LD between these two SNPs should continue to be monitored in each breeding population.

Effect of WUR genotype on disease resilience traits

Genotype counts for the WUR SNP for pigs in the natural disease challenge are in **Table 3.3**. Of the 3126 pigs evaluated, only 25 pigs were homozygous for the favorable allele (GG), consistent with the low frequency of the G allele in the populations evaluated by Boddicker et al. (2014b). Because of these small numbers and indications from Boddicker et al. (2014b) that the G allele shows complete dominance for host response to PRRS, animals with the GG genotype were combined with heterozygous animals for genetic analyses. Results in **Table 3.4** show that

pigs with at least one G allele at the WUR SNP had better performance for a number of traits related to disease resilience. Pigs with at least one G allele were found to have significantly higher challenge nursery average daily gain ($p = 0.02$) and fewer health treatments ($p = 0.05$, 0.22, and 0.01 for treatments in the challenge nursery, the finisher, and across the challenge nursery and finisher, respectively), demonstrating that pigs with the favorable WUR genotypes have higher resilience to a polymicrobial disease challenge. While the effect of WUR genotype was not statistically significant for all traits, numerically, all traits were found to follow the expected trend, with pigs with at least one G allele at the WUR SNP having a more beneficial phenotype. To determine whether most of these effects were the result of PRRS, treatments specific to respiratory problems were analyzed separately. Resulting p-values for the effect of WUR genotype on numbers of respiratory treatments were, however, not significant ($p = 0.66$, 0.26, and 0.54 for treatments in the challenge nursery, the finisher, and across the challenge nursery and finisher, respectively).

Because pigs that are sick are not expected to eat as much as those that are healthy, feed intake under challenge is expected to reflect resilience to disease. Previous studies on the relationship of feed intake with disease resilience include results from Putz et al. (2019), using part of the data used here, who showed that day to day variation in feed intake, feed intake duration, and in the proportion of off-feed days were genetically correlated to mortality and treatment rates under disease. The health of the animal can affect carcass traits as well, as pigs that are not consuming as much feed during the challenge phase are expected to have smaller carcass size. In addition, non-resilient pigs are expected to expend more energy on combating disease rather than gaining weight, therefore, the results in **Table 3.4** reflect the pig's disease resilience ability based on their genotype at the WUR marker.

Conclusions

In conclusion, swine producers can benefit from selection on genotype at the WUR marker. Although the genotype at the WUR marker is not completely consistent with genotype at the putative causative mutation in the *GBP5* gene, differences of the effects of these two SNPs on host response to PRRSV infection were small and not statistically significant. Thus, the WUR SNP, which is present on most commercial SNP panels, can continue to be used to select for improved host response to PRRS. The WUR SNP was also found to be favorably associated with disease resilience in a polymicrobial disease challenge. Pigs with at least one favorable allele had higher growth rate under challenge, fewer health treatments, and a tendency for lower mortality.

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Table 3.1: Concordance (counts) of genotypes at the WUR and GBP5 genetic markers in the combined PHGC trials 1 to 8.

	WUR genotype		
GBP5 Genotype	AA	AG	GG
GG	1011	4	0
GT	6	285	0
TT	1	32	46

Table 3.2: Least squares means (SE) for viral load and weight gain by genotype at the WUR and *GBP5* markers based on data from the PRRS infection trials.

Trait	Marker	Genotype (number of favorable alleles)			p Value
		0	1	2	
Viral Load[1]	WUR	107.72 (0.90)	103.43 (0.97)	104.98 (1.37)	<0.001
	GBP5	107.58 (0.89)	103.07 (0.96)	104.26 (1.20)	<0.001
Weight Gain[2]	WUR	14.00 (0.47)	15.94 (0.50)	15.46 (0.74)	<0.001
	GBP5	14.09 (0.47)	16.05 (0.51)	15.43 (0.65)	<0.001

[1] area under the curve of $\log_2$(viremia) from 0 to 21 days post infection

[2] weight gain from 0 to 42 days post infection (kg)

Table 3.3: Frequency of genotypes at the WUR marker for pigs in the natural disease challenge.

Genotype	Count	Frequency
AA	2699	0.863
AG	402	0.129
GG	25	0.008

Table 3.4: Estimates of the effect of genotype at the WUR SNP (AG+GG versus AA) on response to a polymicrobial disease challenge.

Trait	Number of records	Mean (SD)	Estimate of contrast (AG/GG)-AA	P-value of contrast
Average daily gain (g/day) in:				
Quarantine nursery	3196	280 (0.09)	-1.2	0.76
Challenge nursery	3176	295 (0.18)	18.4	0.02
Finisher	2337	897 (0.13)	8.7	0.26
Average daily feed intake (g/day)	2337	2202 (0.32)	27.5	0.15
Feed conversion ratio (g/g)	2336	2578 (0.20)	13.4	0.26
Residual Feed Intake (g/day)	2336	0.30 (0.13)	-7.0	0.31
Back fat thickness (mm)	2048	17.3 (3.8)	0.2	0.16
Loin depth (mm)	2050	60.2 (6.14)	0.3	0.29
Off-feed days, Duration (%)	2337	0.04 (0.04)	-0.004	0.58
Off-feed days, Feed Intake (%)	2337	0.04 (0.05)	-0.004	0.20
Day-day variation, Duration (min/day)	2337	13.33 (3.78)	0.01	0.11
Day-day variation, Feed Intake (g/day)	2337	501 (0.10)	-5.4	0.39
Lean yield (%)	2045	60.1 (1.71)	-0.2	0.27
Mortality (%) in:				
Challenge nursery	3186	11.4%	-4.1	0.23
Finisher	2829	16.1%	-1.8	0.55
Nursery + finisher	3205	26.3%	-4.3	0.24
Number of health treatments in:				
Challenge nursery (27 days)	3098	1.16 (1.21)	-0.21	0.05
Finisher (100 days)	2295	0.33 (0.62)	-0.06	0.22
Nursery + Finisher (180 days)	2295	1.39 (1.33)	-0.18	0.01
Number of respiratory treatments in:				
Challenge nursery (27 days)	3098	0.55 (0.77)	-0.14	0.66
Finisher (100 days)	2292	0.23 (0.59)	-0.11	0.26
Nursery + Finisher (180 days)	2295	0.36 (0.55)	-0.10	0.54

Supplementary Table 3.1: Frequency of genotypes at the WUR and *GBP5* SNPs in PHGC trials 1 to 8 by genetic source

		WUR		
	GBP5	AA	AG	GG
Trials 1, 2, 3 n=508	GG	364	-	-
	GT	1	125	-
	TT	-	1	15
Trial 4 n=195	GG	159	-	-
	GT	-	20	-
	TT	-	2	4
Trial 5 n=195	GG	134	-	-
	GT	-	50	-
	TT	-	5	4
Trial 6 n=125	GG	113	4	-
	GT	-	1	-
	TT	1	1	-
Trial 7 n=192	GG	84	-	-
	GT	-	65	-
	TT	-	19	23
Trial 8 n=190	GG	157	-	-
	GT	5	24	-
	TT	-	4	-

CHAPTER 4. PROLIFERATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS FROM HEALTHY PIGLETS AFTER MITOGEN STIMULATION AS INDICATORS OF DISEASE RESILIENCE

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Disease resilience refers to productivity of an animal under disease challenge. Given the high biosecurity of pig nucleus herds, traits that can be measured on healthy pigs and that are genetically correlated with disease resilience, i.e. genetic indicator traits, offer a strategy to select for disease resilience. Our objective was to evaluate mitogen stimulation assays on peripheral blood mononuclear cells from young healthy pigs as genetic indicators for disease resilience. Data were from a natural disease challenge in which batches of 60 or 75 naïve Yorkshire x Landrace piglets were introduced every three weeks into a continuous flow barn that was seeded with multiple diseases. Disease resilience traits, including growth, feed intake, and treatment and mortality rates, were recorded on 3136 pigs that were genotyped for 650K SNPs. Peripheral blood mononuclear cells isolated from whole blood collected prior to pathogen challenge from 882 of these pigs from 19 batches were stimulated with five mitogens: concanavalin A (ConA), phytohemagglutinin (PHA), pokeweed mitogen (PWM), lipopolysaccharide (LPS), and phorbol myristate acetate (PMA), and evaluated for counts of proliferated cells after 48, 72, and 96 hrs compared to unstimulated samples (Rest Count). Heritabilities of cell proliferation were estimated using a model with batch as a fixed effect, along with the covariates of entry age, Rest Count, and complete blood count proportions of lymphocytes, monocytes, eosinophils, and basophils, and pen, litter, and animal (with genomic relationships) as random effects. Heritability estimates were highest for ConA (0.29, 0.25, 0.13, and 0.23 at 48, 72, and 96 hrs, and for area under the curve across the three time points, respectively) and were in a similar range for PHA and PMA, but low for PWM and LPS. ConA, PHA, and PMA had moderately high estimates of

genetic correlations with several disease resilience traits that were generally in the expected direction but were not significantly different from zero due to large standard errors. In conclusion, although validation is needed, mitogen stimulation assays, in particular based on ConA, show promise as a genetic indicator trait for disease resilience.

Introduction

Infectious disease remains a substantial problem in the United States swine industry. One such disease is porcine reproductive and respiratory syndrome (PRRS), which is estimated to cost the United States swine industry 664 million US dollars each year (Holtkamp et al., 2013). PRRS is commonly found in swine barns globally and can cause severe reproductive disease, interstitial pneumonia and immune suppression enhancing secondary infections. Porcine circovirus associated disease (PCVAD) was estimated to cost the British swine industry £52.6 million per year prior to the availability of a vaccine in 2008. In addition to PCVAD and PRRS, there are many other viral, bacterial and parasitic pathogens affecting commercial pig production. Common and influential pathogens in the North American industry include *Mycoplasma hyopneumoniae*, swine influenza virus, porcine rotavirus, porcine epidemic diarrhea virus, *Escherichia coli, Lawsonia intracellularis, Streptococcus suis, Brachyspira hyodysenteriae* and Ascaris suum.

A genetic indicator is a trait that is genetically correlated to a trait of interest. A genetic indicator for disease resilience would be a trait that can be measured in the nucleus without infecting the nucleus herd. The two main requirements for a genetic indicator are that it be heritable and it must have a strong genetic correlation with the target trait. One possible class of

genetic indicators for disease resilience is mitogen stimulation assays (MSAs), which are based on the proliferation of peripheral blood mononuclear cells (PBMCs) following stimulation with a mitogen. Mitogens have a similar effect as antigens, resembling pathogen exposure and triggering lymphocyte mitosis immediately after recognition (Ko et al., 1979). Proliferation of PBMCs following mitogen stimulation can be assessed in-vitro to quantify cell-mediated immune response reflecting the potential of an animal's immune response when exposed to infectious pathogens. In the early 2000s, a Disease Resistance Assay for Animals (DRAA) was developed for pigs and evaluated by Pharmagap Inc. (Ottawa, Canada) in conjunction with the Atlantic Swine Research Partnership Inc. and the Canadian Centre for Swine Improvement (CCSI). Although this assay, which was based on stimulation of PBMCs with five mitogens, was never fully validated or made commercially available, significant differences in the immune capacity were reported (Hurnik et al., 2006). In this present study, the same five mitogens were used to stimulate PBMCs isolated from blood of nursery pigs prior to their exposure to a polymicrobial challenge: 1) concanavalin A (ConA), 2) phytohemagglutinin (PHA), 3) pokeweed mitogen (PWM), 4) lipopolysaccharide, and 5) phorbol myristate acetate (PMA). ConA has previously been documented to activate T-lymphocyte division in swine (Lin, 2012), while PHA has been found to activate T-lymphocyte mitosis in rats and humans (Lamers et al., 1999; Piguet et al., 1972), by activating the same subsets of T-lymphocytes as ConA. PWM and PMA are both plant-based mitogens that are known to activate both B- and T-lymphocytes in swine (Paul, et al., 1979; Stepanova et al., 2012). Lastly, LPS is an endotoxin present in all Gram-negative bacteria that acts as a potent stimulator of the innate immune system, and has been shown to activate B-lymphocytes in a wide variety of mammals (Tough, et al., 1997).

Mitogen stimulation assays have been used to measure the genetic basis of porcine immune cell proliferation in two previous studies. Edfors-Lilja et al. (1994) used blood extracted from 124 Yorkshire pigs at 8 weeks of age and reported the heritability of ConA induced immune cell proliferation in-vitro at 48 hours to be 0.38 ±0.21. A similar study was performed by the Flori et al. (2011) but with an expanded set of mitogens including ConA, PMA, and LPS, on blood from 443 Yorkshire pigs at 8 weeks of age, 3 weeks after vaccination against M. *hyopneumoniae*. They estimated the heritability of immune cell proliferation in response to ConA, PMA, and LPS at 48 hours to be 0.36 ±0.20, 0.27 ±0.20, and 0.31 ±0.19.

This study aimed to determine whether MSAs can be used to derive genetic indicators for resilience to polymicrobial disease by estimating 1) the heritability of immune cell proliferation following mitogen stimulation, and 2) phenotypic and genetic correlations among immune cell proliferation assays and with disease resilience traits. Samples and data from a large-scale natural polymicrobial disease challenge study of grow-finish pigs (Putz et al., 2019) were used for this study.

Materials and Methods

Natural Disease Challenge Model

This study was carried out in accordance with the Canadian Council on Animal Care guidelines (CCAC; https://www.ccac.ca/en/certification/about-certification). The protocol was approved by the Protection Committee of the Centre de Recherche en Sciences Animales de Deschambault (CRSAD) and the Animal Care and Use Committee at the University of Alberta (AUP00002227). The project was fully conducted at and overseen by the Centre de développement du porc du Québec (CDPQ) and the herd veterinarian together with project veterinarians. The CDPQ research facility is equipped with tools and staff that allows for

detailed phenotype recording, blood sampling, and strict biosecurity. Data collection for this study started in late 2015 and concluded in early 2019.

The natural disease challenge study consisted of three phases (Putz et al., 2019): (1) a separate quarantine nursery, where the weaned piglets were first placed for around 19 days; (2) a challenge nursery, where the naïve piglets were placed for around 28 days after quarantine and first exposed to disease challenge; (3) an adjoining grow-finishing unit, where the pigs recovered from challenge and were raised until slaughter for on average 100 days. Every three weeks, a new batch of 60 or 75 naïve Yorkshire x Landrace barrows was introduced in the quarantine nursery, alternating between seven members of the PigGen Canada consortium. These pigs were considered naïve, with minimal exposure to disease. After three weeks in the quarantine nursery, the batch of naïve piglets entered into the challenge nursery for 4 weeks, where they were exposed to various pathogens commonly found in commercial swine barns, before entry into the finishing unit. The various pathogens in the natural disease challenge were first established in the barn by introducing infected pigs from nearby farms. Natural disease challenge was maintained through fence line contact of pigs from the new batch with pigs from the previous batch in the challenge nursery. The following viruses were identified in the challenge farm (nursery and grow-finish combined): three strains of the PRRS virus, two serotypes of swine influenza virus, porcine circovirus type 2, and porcine rotavirus A. Bacterial pathogens identified included *Mycoplasma hyopneumoniae*, *Streptoccus suis*, *Glasserella (Haemophilus) parasuis*, *Brachyspira hampsonii*, *Salmonella* spp., *Erysipelothrix rhusiopathiae*, and *Staphylococcus hyicus*. By using natural transmission of common swine pathogens, the natural disease challenge replicated a severe diseased environment in a commercial swine barn. The data used here are from 3139 piglets that were entered the natural disease challenge in 50 batches. All pigs were

genotyped using a commercial 650K SNP panel (Affymetrix), consisting of 658,692 single nucleotide polymorphisms. For more details on the genotyping and imputation of missing genotypes, see Putz et al. (2019).

Mitogen Stimulation Assays

Blood samples used for the MSA were collected in the quarantine nursery, before entry into the challenge nursery, on randomly chosen pigs from batches 13 to 38, for a total of 882 pigs across 18 batches. For batches 13 to 24, blood samples were collected at ~27 days of age, 6 days after arrival in the quarantine nursery. For logistical reasons, for batches 25 to 38, blood samples were collected at ~41 days of age. Complete Blood Count (CBC) data were available on all pigs from blood samples collected at ~27 days of age, as described by Bai et al. (2020). Blood was collected by CDPQ staff in 10 mL vacutainer sodium salt heparinized tubes placed immediately on crushed ice and transported on ice packs to the University of Laval on the same day. PBMCs were purified from the samples on the same day, by gradient centrifugation in 50 mL conical tubes at 400 × g for 10 min to remove the plasma, followed by mixing 1:1 in Hank's balanced salt solution (HBSS) and 12 mL of lymphocyte separation medium (Wisent Inc., St-Bruno, Québec, Canada, #cat: 305-010-CL), then centrifugation at 600 × g for 30 min. The PBMC rings were then transferred into a new 50 mL conical tube with a pasteur pipet and diluted by adding 40 mL of HBSS before centrifugation at 400 × g for 10 min. The cell pellets were gently suspended in 2 mL NH_4Cl solution for 5 min at 37 ℃ to lyse contaminated erythrocytes. Lysis was stopped by adding 20 mL of HBSS and centrifugation at 400 × g for 10 min. Each PBMC sample was then resuspended in 5 mL RPMI1640 (Wisent Inc., St-Bruno, Québec, Canada, #cat: 350045036). Cells were enumerated by using the Trypan blue exclusion procedure (Rao and Otto, 1992). PBMCs were then diluted to a final concentration of 1.33×10^5 cells/mL in

complete RPMI1640 containing 10% of decomplemented exosomes free fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO), glutamine, non-essential amino acids, sodium pyruvate and penicillin/streptomycin (Wisent Inc., St-Bruno, Québec, Canada). The culture was then maintained in a CO_2 incubator (5%) at 37 ^{o}C in a humidified atmosphere.

For the MSA, five mitogens (ConA, PHA, PWM, LPS, and PMA) were strategically chosen to evaluate the proliferation capability of different types of immune cells (**Supplementary Table 4.1)**. The readout of the assay was based on fluorometric estimation of total DNA content of cell populations, which is directly related to the number of cells in the assayed population (Strober et al., 2001). PBMCs were plated in 96 well plates (Costar round bottom) at 20,000 cells/well/150 µl, each row containing PBMCs from one pig. The first and the last rows were empty cells used for background or positive DNA control measurement. The five mitogens were added to PBMCs in columns 7 to 12 at a 4x concentration in 50 µL of complete RPMI. PBMCs in columns 1 to 6 were used as technical replicates for resting or unstimulated cells, by adding 50 µL of complete RPMI. For each sample (columns 1-6), there were six wells for Rest Count and six wells for a mitogen at a specific time point, resulting in six technical replicates per animal per mitogen per time point. The plates were then incubated at 37 ^{o}C in 5% of CO_2 for 48, 72 or 96 hrs. After incubation, the number of cells was quantified by DNA estimation using the Hoechst reagent and a fluorescence plate reader. The 96 well plates were centrifugated for 5 min at 400 × g and the supernatant gently aspirated. The cells were rinsed twice with 200 µL PBS containing Ca^{++} 0.8 mM and Mg^{++} 0.8 mM, before centrifugation. Cells were lysed by adding 100 µL lysis buffer (0.02% SDS in 1X SSC) to each well, except for eight wells in rows 1 and 8, which were reserved for the DNA standard and Blank (four wells each). The plates were then incubated at 37 ^{0}C for 1 h with occasional swirling. Then, 100 µL of 40

µg/mL DNA were added to the four DNA standard wells and 100 µL of 1X SSC buffer to the four blank wells in rows 1 and 8. Then 100 µL of 4 µg/mL Hoechst 33258 reagent in 1X SSC buffer were added to each well. The plate was then incubated on gently agitation at room temperature for 5 min and protected from light by wrapping in aluminum foil, after which the plates could be stored at -70 ^{0}C for a few days. Fluorescence intensity was measured at an excitation wavelength of 355 nm and an emission wavelength of 460 nm or close wavelength depending on the instrument (here Cytofluor, Millipore or Galaxy, BMG labs) Tecan instrument.

Disease Resilience Traits

Disease resilience traits evaluated in the natural disease challenge were average daily gain in the challenge nursery (cNurADG) and in the finisher (FinADG), number of treatments in the challenge nursery (NurTRT), in the finisher (FinTRT), and across the challenge nursery and finisher (ALLTRT), mortality in the challenge nursery (NurMOR), in the finisher (FinMOR), and across the nursery and finisher (ALLMOR). Treatments and mortalities were recorded by CDPQ staff. The number of treatments was a count of the total number of individual treatments that a pig received, which included any drug administered intramuscularly by injection, but excluded group- and batch-level treatments administered via water, feed or parenteral administration. To account for the smaller number of days at risk for pigs that died, the number of treatments was scaled to a standard length of 180 days for number of treatments to average age at slaughter, to 27 days for number of treatments in the challenge nursery, and to 100 days for number of treatments in the finisher. Mortality was also measured as a phenotype for pigs that died in the challenge nursery, the finisher, or across both, with “0” for pigs that survived until the end of that phase, and “1” for pigs that died prior the completing the phase. Subjective health scores were assigned to each pig by trained personnel at four time points: at 5 days after

entry into the quarantine nursery (qHScore1, at ~26 days of age); 19 days after entry into the quarantine nursery (QHScore 2, at ~41 days of age); two weeks after entry into the challenge nursery (cHScore); and six weeks after entry into the finisher (FinHScore). Scores were assigned on a scale of 1 (severe clinical symptoms of disease) to 5 (healthy condition). For more details on the scoring system, please refer to Cheng et al. (2020).

For the finishing traits, FinADG, FinTRT, and ALLTRT, two sets of phenotypes were analyzed. The first set consisted of data on pigs that survived to slaughter (survival data). The second set (expanded data) also included phenotypes on some pigs that died prior to slaughter in the finisher by imputation and expansion of their incomplete phenotypes, as described by Cheng et al. (2020). Only incomplete records that were reliably imputed (based on weight > 0.75, see Cheng et al. (2020)), were included.

Statistical Analyses

For the MSA, two methods were used to adjust cell counts from the stimulated wells for each pig and each mitogen at each time point for cell counts in the unstimulated wells at that time point: 1) Blastogenic Index Score (BIS) computed by dividing the average cell count of the stimulated wells by the average cell count of the non-stimulated well, and 2) by including the average cell count of the non-stimulated wells as a covariate in the model for analysis of the average cell count of the stimulated wells. A BIS score greater than one indicates greater cell proliferation, while a BIS score less than one indicates greater cell death (Han and Dadey, 1978). Data obtained using both approaches were edited by removing extreme outliers, being those that were more than three times the standard deviation away from the mean of the dataset. In total, 292 out of 1320 individual data points were removed for BIS and 171 out of 1320 individual points for stimulated means. After outlier removal, each mitogen at each time point was checked

for normality. Distributions of the BIS and stimulated means data were skewed to the right and, therefore, a log2 transformation was applied prior to analyses.

Data across the three time points were also combined into an Area Under the Curve (AUC) for pigs and mitogens for which all three time points were available. The AUC for each pig and each mitogen was calculated by fitting a quadratic function to log-transformed BIS or stimulated mean data by regression analysis and integrating the resulting quadratic function between 48 and 96 hours. For the AUC for stimulated means, the stimulated mean at each time point was first adjusted for Rest Count based on the estimate of the covariate from the statistical model described in the following. Changes in log-transformed BIS or adjusted stimulated means between time points (deltas) were also computed and analyzed for each mitogen (delta = 72 – 48 hrs, 96 – 72 hrs, and 96 – 48 hrs).

The following univariate model was used to estimate the heritability of BIS for each mitogen at each time point, using ASReml 4.0 (Gilmour et al., 2009):

$$Y_{ijklm} = \mu + Batch_j + b_1 * Age_i + b_2 * Lymphocytes_i + b_3 * Monocytes_i + b_4 * Eosinophils_i + b_5 * Basophils_i + Animal_i + Sow_k + Pen_{l(j)} + Plate_{m(j)} + e_{ijklm}$$

where Y_{ijklm} = the $\log_2$(BIS) for animal i for a given mitogen at a given time point; Batch = fixed effect of batch; Age = the age at entry into the quarantine nursery; Lymphocytes, Monocytes, Eosinophils, Basophils = the proportion of each immune cell type from the CBC data; Animal = random animal genetic effect of the i_{th} individual, assumed distributed ~N(0, $\mathbf{G}\sigma_g^2$), with **G** equal to the genomic relationship matrix constructed using SNP genotypes, with pigs from different companies assumed unrelated, and σ_g^2 equal to the genetic variance; Sow = sow or litter effect; Pen = random effect of pen nested within Batch (50 levels); Plate = random

effect of plate nested within batch (146 levels); e = residual. For analysis of the Stimulated Mean data, the log2-transformed cell count of non-stimulated wells was added to the model as a covariate. Proportions of cell types from the CBC data were used as covariates to account for any possible impact of cell proportions on the MSA. Although none of the covariates were significant, they were conservatively left in the model because they only took up 4 degrees of freedom. Fitting separate covariates for MSA conducted on blood collected at ~27 versus ~41 days of age, with CBC always taken at the ~27 day time point, did not result in significant differences and, therefore, only one covariate per cell type was fitted in the final model. The proportion of neutrophils and of 'large unidentified cells', as obtained from the CBC data, were not included in the model because the proportion of neutrophils was highly correlated with the proportion of lymphocytes (r=0.91), while the 'large unidentified cells' were determined to be mostly agglutinated cells. The univariate analyses were used to estimate the narrow sense heritability (h^2) for each MSA trait, as well as variance due litter effects as a proportion of phenotypic variance (c^2). Phenotypic variance was estimated as the sum of estimates of variances due to animal genetics, sow, and residuals.

Bivariate analyses were conducted to obtain four sets of genetic and phenotypic correlations. The first set consisted of correlations between the BIS and the corresponding stimulated mean phenotypes, to evaluate the impact of the two approaches to account for Rest Count. The second set consisted of correlations between time points and the AUC for the same mitogen for both the BIS and stimulated mean data. The third set consisted of correlations among different mitogens at the same time point and the AUC for both the BIS and stimulated mean data. The fourth set estimated consisted of genetic and phenotypic correlations of the MSA traits with each of the disease resilience traits evaluated. Correlations were estimated using bivariate

models in ASReml 4.0, using the model described above for the MSA traits and models described in Cheng et al. (2020) for disease resilience traits. For the latter, resilience data from all 3139 pigs across 50 batches were used, using their genomic relationships with pigs with MSA data.

The first set of correlations could not be estimated using ASReml 4.0 (no convergence) and were, instead, estimated by analyzing the sum of BIS and stimulated mean as a phenotype. For these analyses, the stimulated mean data were adjusted for the covariate Rest Count using the regression coefficient that was obtained from the univariate analysis of each trait. The sum of each BIS phenotype with its corresponding adjusted stimulated mean phenotype was then analyzed as a single trait using the univariate model described above to estimate its phenotypic and genetic variance. The covariance (genetic or phenotypic) between the BIS and stimulated mean phenotypes was then estimated by rearranging the formula of a sum as:

$$\mathrm{Var}(\mathrm{BIS} + \mathrm{Stimmean}) = \mathrm{Var}(\mathrm{BIS}) + \mathrm{Var}(\mathrm{StimMean}) + 2\mathrm{Cov}(\mathrm{BIS}, \mathrm{StimMean})$$

$$\mathrm{Cov}(\mathrm{BIS}, \mathrm{StimMean}) = \frac{\mathrm{Var}(\mathrm{BIS} + \mathrm{StimMean}) - \mathrm{Var}(\mathrm{BIS}) - \mathrm{Var}(\mathrm{StimMean})}{2}$$

Estimates of genetic and phenotypic variances for the individual phenotypes were as obtained from the univariate analyses.

Results

Basic Statistics of MSA Data

Descriptive statistics of the MSA assay results are presented in **Table 4.1**. For resting cell count, ConA showed a general increase from 48 to 96 hrs. This proliferation in unstimulated cells is unexpected, since growth factors in the blood sample are in limited supply. For the other

four mitogens, the average resting cell count either went down or was relatively stable between 48 and 96 hours, as expected, since cells expend energy to maintain homeostasis in a resting state. For the stimulated wells, there were large numerical changes and observable patterns between 48 and 96 hrs. For PWM, the largest mean cell count was at 48 hrs, while for ConA, the largest count was at 72 hrs. For PHA, LPS, and PMA, the largest count was at 96 hrs. This variety in trends between mitogens becomes more complex when considering the changes between two individual time points. The within-sample coefficient of variation was between 13 and 17% for the resting cell counts but generally higher, up to 29% for the stimulated cell counts.

Descriptive statistics of the log-transformed BIS scores and stimulated mean phenotypes are presented in **Table 4.2**. The means of the stimulated mean data were fairly similar across the five mitogens, ranging from 12.0 to 12.7 for individual time points, from 1671 to 1759 for AUC, and from -0.4 to 0.2 for individual deltas. This pattern of consistency was not evident for BIS scores. Notably, the means for individual time points and for AUC were substantially larger for ConA, PHA, and PMA than for PWM and LPS. In addition, for each mitogen, the largest average BIS was at 72 hrs.

Heritability of MSA Phenotypes

Heritability for each MSA phenotype was estimated for each mitogen for both the BIS and stimulated mean data and are shown in **Table 4.2**. In general, estimates of heritability were numerically very similar for BIS and stimulated means and, therefore, only estimates for BIS will be discussed in the remainder of this section. Estimates of litter effects were all very small for all MSA phenotypes **(Table 4.2)**, with most values being zero, suggesting that the environment provided by the sow had very limited impact on the MSA phenotypes.

Estimates of heritability at individual time points were moderately high (0.19 to 0.28) for ConA and PHA at all three time points and for PMA at 72 hrs (0.29) but low for PWM and LPS across all time points (<0.10) and for PMA at 48 (0.12) and 96 hrs (0.04). For all mitogens, with the exception of LPS, the largest estimate of heritability was at 72 hrs. This pattern was consistent with the largest BIS scores being at 72 hrs for most mitogens.

Heritability estimates for AUC were similar to the highest estimate across time points for each mitogen, except for PHA, for which the estimate of heritability of AUC was higher than at any of the three individual time points. The delta phenotypes showed no clear patterns in heritability estimates. For ConA, PWM, and PMA, change from 48 to 72 hrs had heritability estimates that were higher or as high as the most heritable individual time point. Estimates of heritabilities for most other delta phenotypes were as large as or smaller than for individual time points.

Correlations between BIS and Stimulated Mean Phenotypes

Estimates of genetic and phenotypic correlations between BIS and their corresponding simulated mean phenotypes are in **Table 4.2**. These estimates provide insight into the effect of these two methods of accounting for the count of unstimulated wells. Because of lack of convergence of estimates with ASReml, these correlations were calculated based on an alternate method that does not restrict estimates to the parameter space, resulting in some estimates to be greater than 1. In general, estimates of phenotypic correlations between BIS and the corresponding stimulated mean phenotypes were high, whereas estimates of genetic correlations were also high but very variable, reflecting the large standard errors for estimates of genetic correlations. The average estimates of genetic correlations between the BIS and corresponding stimulated mean phenotypes within a mitogen were 0.86, 1.11, 0.67, 1.27, and 1.20 for ConA,

PHA, PWM, LPS, and PMA, respectively. These high genetic correlations suggest that selecting on either BIS or stimulated mean would have similar effects.

Correlations Among Time Points within the Same Mitogen

Estimates of genetic correlations between time points and the AUC for the same mitogen are in **Table 4.3**, while corresponding phenotypic correlations are in **Supplementary Table 4.2**. For both the BIS and stimulated mean phenotypes, the magnitude of all estimated genetic correlations was found to be positive. Some estimates were large, but all estimates had substantial standard errors. When considering these large standard errors, it is notable that for ConA and PHA, estimates based on BIS were similar to corresponding estimates based on stimulated means for each pair of time points. This was as expected, as the BIS and stimulated mean phenotypes were found to have high genetic correlations (**Table 4.2**).

Correlations Among Mitogens at the Same Time Point

Estimates of genetic among mitogens at the same time point are in **Table 4.3** and corresponding phenotypic correlations in **Supplementary Table 3.3**. Similar to the genetic correlations among time points within the same mitogen, several genetic correlations could not be estimated, especially those that involved PWM and LPS phenotypes. All estimated genetic correlations were positive and some were some high positive (> 0.90), but with large standard errors. When considering the standard errors, genetic correlation estimates between mitogens based on BIS were similar to those based on stimulated mean. Genetic correlation estimates between ConA and PHA for corresponding MSA phenotypes were consistently strong, which was as expected since these two mitogens activate similar cell types.

Correlations of MSA with Disease Resilience Traits

Descriptive statistics of the evaluated performance and resilience traits are in Cheng et al. (2020). Reasons for treatments and mortalities and their frequencies are summarized in **Supplementary Table 4.4**. Infection and disease were responsible for nearly all of the prescribed treatments and mortalities causes. Respiratory distress accounted for 956 (27.9%) of treatment reasons and 187 (19.2%) of mortality reasons. Non-infectious causes only accounted for a small portion of reasons.

Estimates of genetic correlations of MSA phenotypes with disease resilience traits are in **Figure 4.1** and corresponding phenotypic correlations are in **Supplementary Tables 4.5** for BIS and in **Supplementary Table 4.6** for stimulated means. Heat maps displaying genetic and phenotypic correlations of MSA phenotypes with disease resilience traits are in **Supplementary Figure 4.2**. Estimates of phenotypic correlations were low, suggesting limited opportunities to predict the resilience of an individual pig based on its MSA phenotypes. For genetic correlations, estimates are only presented for ConA, PHA, and PMA because the low heritabilities for PWM and LPS resulted in genetic correlation estimates with very large standard errors or could not be estimated. Even the estimates for the ConA, PHA, and PMA had large standard errors and none were statistically significantly different from 0 ($p>0.05$) based on the likelihood ratio test (King, 2020). Nevertheless, several MSA phenotypes showed consistent trends in estimates across time points and across resilience traits in the expected direction, and are described in more detail below. For the finisher traits, estimates of genetic correlations were fairly similar between the survival and expanded data (**Supplementary Figure** 4.**1**). Therefore, only estimates for the survival data will be described in the following.

Growth rate: Estimates of genetic correlations of BIS phenotypes with growth rate in the quarantine nursery (qNurADG), the challenge nursery (cNurADG), and in the finisher (FinADG) are shown in **Figure 4.1**. The overall trend of estimates genetic correlations with growth rate in the quarantine nursery was found to be ambivalent. Associations of MSA phenotypes with growth rate in the quarantine nursery were expected to be weak since the expression of disease is expected to be minimal at this stage. Some genetic correlation estimates with qNurADG differed substantially when based on BIS versus stimulated mean data, e.g. ConA and PHA at 48 hrs, 72 hrs, and 96 hrs. Additionally, estimates for ConA were positive at each time points based on BIS but negative when based on stimulated mean. Standard errors were large. Estimates with AUC were negative but weak for all three mitogens. Genetic correlation estimates with deltas were mostly positive but generally weak.

Estimates of genetic correlations with growth rate in the challenge nursery were positive, as expected, for ConA and PMA phenotypes at individuals time points and AUC. Corresponding estimates for PHA were weak and variable in sign. The delta phenotypes had weak and variable genetic correlation estimates with cNurADG. Differences between estimates based on BIS versus stimulated means were generally small, except for PHA at 48 hrs and for ConA for delta 96–48.

Estimates of genetic correlations of MSA phenotypes at individual time points and for AUC with growth rate in the finisher were generally weak. Genetic correlations with AUC based on stimulated means could not be estimated. Estimates of genetic correlations of FinADG with the delta MSA phenotypes were positive for delta 72-48 and delta 96-72, but negative for delta 96-48. PMA phenotypes had the strongest estimates of genetic correlations with FinADG among the three mitogens, but with large standard errors. Differences between estimates based on BIS and stimulated means were minimal, except for PMA at 72 hrs and 96 hrs. Genetic correlations

with growth rate in the finisher were also estimated using pigs from the expanded dataset (**Supplementary Figure 4.1**). Generally, differences between estimates obtained using the survivor and expanded datasets were minimal.

Treatment Rates: Estimates of genetic correlations with numbers of treatments in the challenge nursery were generally negative for MSA phenotypes at individual time points and for AUC. For the delta MSA phenotypes, genetic correlation estimates were consistently negative for all three mitogens. Differences between estimates based on BIS and stimulated means were small, except for PMA at 48 hrs and 96 hrs, for which the sign of the estimate differed between the two.

The majority of genetic correlations with number of treatments in the finisher could not be estimated because the heritability of this resilience trait was very small. Correlations that could be estimated had extremely high standard errors and showed no clear trend. Estimates of genetic correlations of the number of treatments across the challenge nursery and finisher with MSA phenotypes generally followed similar trends as observed for treatments in the challenge nursery. However, the patterns observed for the delta phenotypes for number of treatments in the nursery were not evident across the nursery and finisher. Genetic correlations for number of treatments in the finisher and across the challenge nursery and finisher were also estimated using the expanded dataset (**Supplementary Figure 4.1**). Comparisons could not be made for number of treatments in the finisher because of lack of convergence. For number of treatments across the challenge nursery and finisher, estimates tended to be smaller for the expanded data set and differences were substantial for a number of MSA phenotype, in particular for PHA phenotypes.

Mortality: For mortality in the challenge nursery, estimates of genetic correlations with MSA phenotypes generally followed expected trends, with negative estimates for individual time

points and AUC. The delta's estimates for PMA followed the same trend observed of number of treatments in the challenge nursery, i.e. increasing from negative estimates for delta 72-48 to positive estimates for delta 96-48. Opposite trends were, however, observed for estimates for ConA and PHA, i.e. decreasing from positive for delta 72-48 to negative for delta 96-48. Differences between estimates based on BIS and stimulated mean were substantial for ConA at the individual time points, but were small for the ConA and for most MSA phenotypes to PHA and PMA.

For mortality in the finisher, estimates of genetic correlations with MSA phenotypes at individual time points were similar to those observed for mortality in the nursery, but estimates with AUC were much weaker for mortality in the finisher than in the nursery. There were no clear trends for correlations with the delta phenotypes, except that estimates with delta 96-72 were large positive but with very large standard errors. Differences in estimates based on BIS and stimulated means were small for most MSA phenotypes, except for PHA and PMA at 48 hrs,

Patterns in estimates of genetic correlations of mortality across the nursery and finisher with MSA phenotypes at individual time points were very similar to those for mortality in the nursery and in the finisher but with a few more positive estimates. Estimates for delta phenotypes tended to be similar to those for mortality in the finisher, rather than to estimates for mortality in the nursery.

Health scores: Estimates of genetic correlations between MSA phenotypes with health scores are in **Figure 4.1**, with corresponding phenotypic correlations in **Supplementary Table 4.7**. For the two health scores collected in the quarantine nursery, all pigs scored either as 4 or 5 (Cheng et al., 2020) and there were no expected trends in the correlation estimates because of the high health status of at this stage. While there were no clear trends in estimates for the first

health score, the second health score tended towards positive genetic correlation estimates for individual time points and in particular for AUC. Additionally, for the delta phenotypes, all estimates were positive for PHA and negative for PMA. Genetic correlation estimates for the second health score had large standard errors because the heritability estimate for this health score was very low (0.00). Differences between estimates based on BIS and stimulated mean were small for both health scores, although there were a few instances where the estimates changed sign.

Estimates of genetic correlations for health scores in the challenge nursery and finisher were nearly all positive for all MSA phenotypes and across all three mitogens. Some estimates were strong but all had large standard errors. Generally, differences between estimates based on BIS and stimulated mean were small, but with some exceptions.

Discussion

Commercial pigs often face a wide variety of infectious swine diseases that are generally not observed in the nucleus herds. Selection for disease resilience is difficult due to the structure of the US swine production system and the lack of a DNA marker for marker-assisted selection. Identification of a genetic indicator trait for increased disease resilience that can be measured on young healthy pigs can be a pragmatic strategy that would facilitate the production of resilient commercial pigs while optimizing the efficiency of the swine industry, without requiring swine breeders to infect nucleus animals with disease or collect disease data in commercial herds.

In this study, we measured the proliferation of PMCs from young healthy pigs after mitogen stimulation as possible genetic indicators for disease resilience. Through the use of an

MSA, we identified three mitogens (ConA, PHA, and PMA) whose in vitro PBMC stimulation phenotypes had moderate estimates of heritabilities. ConA, PHA, and PMA are commonly used as mitogens in in-vitro experiments to stimulate specific pathways and assess the immune response of an organism (Lin, 2012, Lamers et al., 1999, Paul, et al., 1979, Stepanova et al., 2012, Tough, et al., 1997). Heritability is an important genetic parameter that determines to what extent an animal's response is affected by genetics and, therefore, large heritabilities will lead to a substantial response of a trait to selection (Lush, 1937).

Our estimates of heritability for ConA at 48 hrs (0.24 ±0.08) are consistent with the findings from that of Edfors-Lilja et al. (2011) (0.38 ±0.21) and Flori et al. (2011) (0.36 ±0.20). However, there were numerical differences between our heritability estimates for PMA and LPS at time 48 (0.12 ±0.08 and 0.00 ±0.00) and those estimated by Flori et al. (2011) (0.27 ±0.20 and 0.31 ±0.19). These differences can be attributed to many factors. First, Flori et al. (2011) response to mitogens three weeks after vaccination for M*ycoplasma hyopneumoniae.* Second, sample size in the study of Flori et al. (2011) was roughly half of ours, which resulted in standard errors for estimates of heritability to be roughly twice the size of ours. Third, different breeds were used in the two studies, Large White by Flori et al. (2011) versus Landrace x Yorkshire crossbreds in our study.

For the three mitogens that resulted in sizeable estimates of heritabilities (ConA, PHA, and PMA), estimates of genetic correlations of MSA phenotypes with disease resilience followed expected trends in most cases. The overall trend for the estimates of genetic correlations between growth rate and health scores, with MSA traits was expected to be positive. This is because a faster response to mitogen induced immune cell proliferation is indicative of higher disease resilience. Therefore, animals with a greater response to mitogen stimulation, are expected to

gain weight faster than sick pigs and have higher health scores. For traits that were taken prior to the disease challenge, such as growth rate and health scores, there were no expected trends or patterns since these health scores were assigned prior to the disease challenge. Estimates of genetic correlations of treatment and mortality with MSA traits were expected to be negative. Animals with a greater response to mitogen stimulations are expected to require fewer treatments and result in fewer mortalities. Although one would expect number of treatments and health scores to be highly correlated, health scores are more objective because the choice whether or not to treat is removed. For instance, some sick pigs may have lower health scores but they are not sick enough to treat.

Although none of the individual genetic correlation estimates were significantly different from zero, similar trends across mitogens and disease resilience traits suggest that at least the direction of the genetic correlations with disease resilience traits is as expected. This suggests that selection for higher MSA response to these three mitogens is expected to result in greater growth rate, higher health scores, fewer health treatments, and lower rates of mortality under a polymicrobial challenge. Biologically, higher rates of peripheral blood mononuclear cell proliferation in the blood of resilient pigs may coordinate a more efficient and responsive adaptive immune response, effectively expediting the expression of peripheral blood mononuclear cells and thus, mitigating the antagonistic physiological consequences of pathogenic infection.

In order to determine which of the five mitogens would be the best to use for an MSA as a genetic indicator trait for disease resilience, we first considered estimates of heritability for the MSA. Since LPS and PWM MSA phenotypes had low estimates of heritability, we removed these two mitogens from consideration. In terms of heritability, there are no notable advantages

of using any of the remaining three mitogens over one another, although heritability estimates for ConA were moderate across all three time points, while PHA and PMA had lower estimates at 48 and 96 hrs, especially PMA. AUC and delta phenotypes did not provide higher estimates of heritabilities for these three mitogens. After observing trends in heritability, visualizing estimates of genetic correlations of the MSA with disease resilience in **Figure 4.1** and in **Supplementary Figure 4.2** provided more insight into the suitability of the different MSA phenotypes as indicator traits for disease resilience. Based on these figures, MSA phenotypes based on ConA generally showed more consistent estimates in the expected direction across time points. Although PMA resulted in the numerically highest genetic correlation estimates, estimated for PMA and PHA were not as consistent relative to expectations and showed large standard errors. When comparing the MSA phenotypes of ConA between BIS and stimulated mean, there were few substantial differences and genetic correlation trends were generally the same between the two. Therefore, choosing either BIS or stimulated mean may yield no noticeable difference. Differences between expanded and survivor datasets were also small. However, Cheng et al. (2020) recommend using the expanded dataset because it reduces selection biases and includes more pigs into the analysis (Cheng et al., 2020). Based on these results, ConA showed the most promise as a genetic indicator for disease resilience. However, further studies are required to not only validate ConA's potential as an indicator, but also to determine which mitogen time point may be the best.

Conclusions

Swine producers can benefit from using MSA of PBMCs isolated from the blood of young healthy pigs as genetic indicators of disease resilience. Estimates of heritability of

response to Concanavalin A were moderately high at each of the three time points after stimulation and across the three time points based on area under the curve. In most cases, the heritability estimates of phenotypes extracted from stimulation with phytohemagglutinin and phorbol myristate acetate were also moderately heritable, while those obtained from stimulation with pokeweed mitogen and lipopolysaccharide were lowly heritable. However, estimates of genetic correlations of response to Concanavalin A with disease resilience traits were more consistent across time and traits and followed expectations better than those of response to the other two heritable mitogens. between each mitogen with several disease resilience traits were also estimated, where correlations using ConA consistently followed expected trends with generally smaller standard errors than PHA and PMA. Thus, although these results should be validated to overcome large standard errors of estimates, swine breeders can benefit from utilizing in vitro MSA to ConA, using blood samples collected from young healthy pigs to select for increased disease resilience.

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Table 4.1: Mean and within-sample coefficients of variation (CV) across six replicates for the number of live cells in unstimulated (restcount) and stimulated samples (Stim Mean).

			Rest Count		Stim Mean	
Mitogen	Time	Number of Samples	Mean	CV (%)	Mean	CV (%)
Concanavalin A	48	860	4971.9	15.0	6700.0	21.5
	72	873	5590.9	12.9	8313.7	21.3
	96	871	5719.7	13.1	6679.8	16.3
Phytohemagglutinin	48	860	5814.7	13.1	6548.6	14.0
	72	872	5029.5	13.3	7289.5	22.6
	96	855	4224.8	14.0	7640.8	24.6
Poke Weed Mitogen	48	866	4003.3	15.4	8482.5	28.7
	72	858	3970.6	15.0	5091.5	19.6
	96	855	4052.4	15.7	4578.6	15.6
Lipopolysaccharide	48	859	3999.7	15.2	7116.5	25.0
	72	862	3811.4	15.9	6768.2	24.8
	96	856	4043.4	15.3	7608.4	26.0
Phorbol Myristate Acetate	48	854	4063.1	16.1	4737.9	18.6
	72	868	4039.1	16.0	4412.2	15.5
	96	869	4201.6	16.8	6856.8	24.9

Table 4.2: Basic statistics (mean and SD) and estimates of heritability (h^2) and litter effects (c^2) (SE in parentheses) for log(2) transformed BIS and stimulated mean phenotypes for each of 5 mitogens at 3 time points (48, 72, and 96 hrs after stimulation), and for area under the curve (AUC) and for changes in phenotypes between time points (72-48, 96-48, and 96-72), as well as estimates of phenotypic (r_p) and genetic (r_g) correlations between corresponding BIS and stimulated mean phenotypes.

		BIS					Stimulated Mean					Correlation	
Mitogen	Time	Number of pigs	Mean	SD	h^2 (SE)	c^2 (SE)	Number of pigs	Mean	SD	h^2 (SE)	c^2 (SE)	r_p	r_g
Concanavalin A	48	860	0.30	0.37	0.24 (0.08)	0.00 (0.00)	863	12.4	0.90	0.30 (0.09)	0.00 (0.00)	0.89	0.62
	72	873	0.70	0.63	0.27 (0.09)	0.00 (0.00)	868	12.6	0.89	0.28 (0.10)	0.00 (0.00)	0.97	1.04
	96	871	0.66	0.61	0.23 (0.10)	0.00 (0.00)	866	12.5	0.83	0.17 (0.10)	0.04 (0.05)	0.89	0.93
	AUC	850	43.43	36.45	0.24 (0.09)	0.00 (0.00)	859	1743	52.53	0.25 (0.10)	0.01 (0.05)	1.04	1.10
	72-48	860	0.39	0.56	0.34 (0.10)	0.00 (0.00)	861	-0.4	0.78	0.37 (0.10)	0.00 (0.00)	1.21	0.71
	96-72	871	-0.03	0.53	0.13 (0.09)	0.00 (0.00)	865	0.0	0.75	0.13 (0.09)	0.00 (0.00)	1.04	0.82
	96-48	859	0.35	0.56	0.21 (0.10)	0.01 (0.05)	860	0.4	0.93	0.19 (0.09)	0.00 (0.00)	0.89	0.81
Phytohemagglutinin	48	860	0.48	0.43	0.19 (0.09)	0.02 (0.05)	862	12.7	1.02	0.07 (0.09)	0.02 (0.06)	0.84	1.86
	72	872	0.85	0.73	0.28 (0.09)	0.00 (0.00)	866	12.7	0.97	0.33 (0.10)	0.00 (0.00)	0.89	1.07
	96	855	0.71	0.76	0.22 (0.10)	0.04 (0.05)	849	12.5	0.95	0.22 (0.10)	0.04 (0.05)	0.90	0.95
	AUC	836	54.85	44.89	0.34 (0.09)	0.00 (0.00)	857	1759	60.78	0.28 (0.10)	0.00 (0.00)	0.90	0.75
	72-48	859	0.37	0.58	0.13 (0.08)	0.00 (0.00)	857	-0.4	0.90	0.13 (0.08)	0.00 (0.00)	0.81	1.24
	96-72	854	-0.15	0.55	0.14 (0.07)	0.00 (0.00)	846	0.1	0.63	0.10 (0.07)	0.00 (0.00)	1.01	0.89
	96-48	842	0.23	0.62	0.18 (0.09)	0.01 (0.06)	845	0.2	1.08	0.18 (0.9)	0.01 (0.06)	0.74	1.01
Poke Weed Mitogen	48	866	0.14	0.29	0.00 (0.00)	0.01 (0.05)	863	12.3	0.91	0.00 (0.09)	0.01 (0.06)	0.96	1.44
	72	858	0.32	0.32	0.10 (0.08)	0.00 (0.00)	864	12.2	0.58	0.15 (0.09)	0.00 (0.00)	0.83	0.62
	96	855	0.20	0.37	0.09 (0.08)	0.00 (0.00)	865	12.1	0.55	0.07 (0.08)	0.00 (0.00)	0.82	0.70
	AUC	848	20.11	19.14	0.03 (0.06)	0.00 (0.00)	848	1684	37.33	0.09 (0.08)	0.00 (0.00)	0.74	2.10
	72-48	856	0.18	0.37	0.12 (0.08)	0.00 (0.00)	856	-0.2	0.82	0.13 (0.08)	0.00 (0.00)	0.96	0.83
	96-72	856	-0.12	0.42	NE	NE	856	0.1	0.59	NE	NE	NE	NE
	96-48	848	0.06	0.38	0.03 (0.07)	0.00 (0.00)	855	0.1	1.02	0.02 (0.07)	0.00 (0.00)	0.65	0.21
Lipopolysaccharide	48	859	0.13	0.23	0.00 (0.00)	0.00 (0.00)	863	12.3	0.88	0.00 (0.09)	0.00 (0.00)	NE	NE
	72	862	0.17	0.27	0.05 (0.07)	0.00 (0.00)	868	12.1	0.52	0.10 (0.09)	0.00 (0.00)	1.18	0.18
	96	856	0.13	0.29	0.07 (0.09)	0.03 (0.06)	864	12.0	0.52	0.00 (0.09)	0.04 (0.06)	1.34	2.02
	AUC	834	11.11	16.52	0.03 (0.06)	0.00 (0.00)	854	1671	33.93	0.00 (0.09)	0.08 (0.05)	0.60	0.41
	72-48	849	0.04	0.27	0.01 (0.08)	0.00 (0.00)	849	0.0	0.72	0.02 (0.08)	0.00 (0.00)	1.71	-0.43
	96-72	846	-0.03	0.29	NE	NE	846	0.0	0.55	0.01 (0.08)	0.00 (0.00)	NE	NE
	96-48	844	0.01	0.29	0.00 (0.00)	0.04 (0.05)	844	0.0	0.99	0.00 (0.00)	0.04 (0.05)	1.28	4.17
Phorbol Myristate Acetate	48	854	0.39	0.42	0.12 (0.08)	0.00 (0.00)	864	12.5	0.89	0.10 (0.09)	0.00 (0.01)	1.04	0.86
	72	868	0.66	0.62	0.29 (0.09)	0.01 (0.05)	867	12.5	0.96	0.22 (0.09)	0.01 (0.05)	1.19	1.29
	96	869	0.55	0.70	0.04 (0.06)	0.00 (0.00)	867	12.4	0.89	0.03 (0.06)	0.00 (0.00)	0.97	1.48
	AUC	851	43.51	37.35	0.27 (0.10)	0.04 (0.06)	861	1716	61.37	0.30 (0.10)	0.00 (0.00)	0.78	1.21
	72-48	852	0.27	0.53	0.26 (0.09)	0.00 (0.00)	861	-0.3	0.76	0.25 (0.09)	0.00 (0.00)	1.13	1.51
	96-72	865	-0.11	0.56	0.05 (0.06)	0.00 (0.00)	867	0.1	0.83	0.04 (0.06)	0.00 (0.00)	1.04	1.22
	96-48	852	0.15	0.64	0.13 (0.09)	0.01 (0.05)	861	0.2	0.90	0.13 (0.09)	0.01 (0.05)	1.03	0.86

48: 48 hrs; 72: 72 hrs; 96: 96 hrs; AUC: Area Under the Curve; 72 – 48: difference between 72 hrs and 48 hrs; 96 – 72: difference between 96 hrs and 72 hrs; 96 – 48: difference between 96 and 48 hrs; NE: not estimable

Table 4.3: Estimates of genetic correlations for BIS (below diagonal) and Stimulated Means (above diagonal) (SE in parentheses) between time points (48, 72, or 72 hrs after stimulation) and for area under the curve (AUC) for each of five mitogens.

Concanavalin A	48	72	96	AUC
48		0.42 (0.21)	0.53 (0.23)	0.87 (0.12)
72	0.24 (0.25)		0.11 (0.66)	NE
96	0.51 (0.22)	0.78 (0.15)		0.82 (0.14)
AUC	0.41 (0.22)	NE	0.90 (0.10)	

Phytohemagglutinin	48	72	96	AUC
48		NE	0.73 (0.24)	NE
72	0.86 (0.14)		0.91 (0.09)	NE
96	0.68 (0.17)	0.83 (0.11)		0.92 (0.12)
AUC	0.36 (0.22)	NE	0.83 (0.10)	

Poke Weed Mitogen	48	72	96	AUC
48		0.53 (0.31)	0.45 (0.55)	0.56 (0.69)
72	NE		0.41 (0.80)	0.13 (0.91)
96	NE	0.67 (0.43)		NE
AUC	NE	NE	NE	

Lipopolysaccharide	48	72	96	AUC
48		0.27 (0.41)	0.29 (0.44)	0.93 (0.25)
72	NE		0.44 (0.41)	0.09 (0.84)
96	NE	NE		0.89 (0.56)
AUC	NE	NE	NE	

Phorbol Myristate Acetate	48	72	96	AUC
48		0.78 (0.24)	0.24 (0.63)	NE
72	0.65 (0.21)		NE	0.36 (0.46)
96	0.46 (0.66)	NE		0.21 (0.45)
AUC	0.76 (0.14)	NE	NE	

48: 48 hrs; 72: 72 hrs; 96: 96 hrs; AUC: Area Under the Curve

NE: not estimable.

Table 4.4: Estimates of genetic correlations for BIS (below diagonal) and Stimulated Mean (above diagonal) data (SE in parentheses) between mitogens at a given time point (48, 72, or 72 hrs after stimulation) and for area under the curve (AUC).

Trait	Con A 48	PHA 48	PWM 48	LPS 48	PMA 48
Con A 48		0.96 (0.72)	NE	0.00 (0.00)	NE
PHA 48	0.65 (0.19)		NE	0.00 (0.00)	0.25 (0.79)
PWM 48	NE	NE		0.00 (0.00)	NE
LPS 48	NE	NE	NE		NE
PMA 48	NE	0.73 (0.32)	NE	NE	

Trait	Con A 72	PHA 72	PWM 72	LPS 72	PMA 72
Con A 72		0.74 (0.13)	0.00 (0.00)	0.40 (0.29)	0.92 (0.19)
PHA 72	0.55 (0.17)		0.00 (0.00)	0.77 (0.27)	0.89 (0.16)
PWM 72	0.00 (0.00)	0.00 (0.00)		NE	NE
LPS 72	0.65 (0.70)	NE	NE		0.53 (0.34)
PMA 72	0.71 (0.16)	0.91 (0.12)	0.47 (0.31)	0.67 (1.17)	

Trait	Con A 96	PHA 96	PWM 96	LPS 96	PMA 96
Con A 96		0.64 (0.19)	0.87 (0.33)	0.65 (1.19)	NE
PHA 96	0.62 (0.16)		0.44 (0.83)	NE	NE
PWM 96	0.28 (0.50)	0.88 (0.37)		0.45 (0.71)	0.57 (0.74)
LPS 96	0.60 (0.47)	0.13 (0.43)	NE		NE
PMA 96	NE	0.34 (0.72)	NE	NE	

Trait	Con A AUC	PHA AUC	PWM AUC	LPS AUC	PWM AUC
Con A AUC		0.74 (0.12)	0.96 (0.21)	0.15 (0.44)	0.87 (0.17)
PHA AUC	0.60 (0.15)		0.98 (0.11)	0.74 (0.36)	0.78 (0.11)
PWM AUC	0.99 (0.89)	NE		0.92 (0.50)	NE
LPS AUC	0.66 (0.94)	0.04 (0.74)	NE		0.92 (0.50)
PMA AUC	0.76 (0.16)	0.86 (0.11)	NE	NE	

Con A: Concanavalin A; PHA: Phytohemagglutinin; PWM: Poke Weed Mitogen; LPS: Lipopolysaccharide; PMA: Phorbol Myristate Acetate; AUC: Area Under the Curve

NE: not estimable.

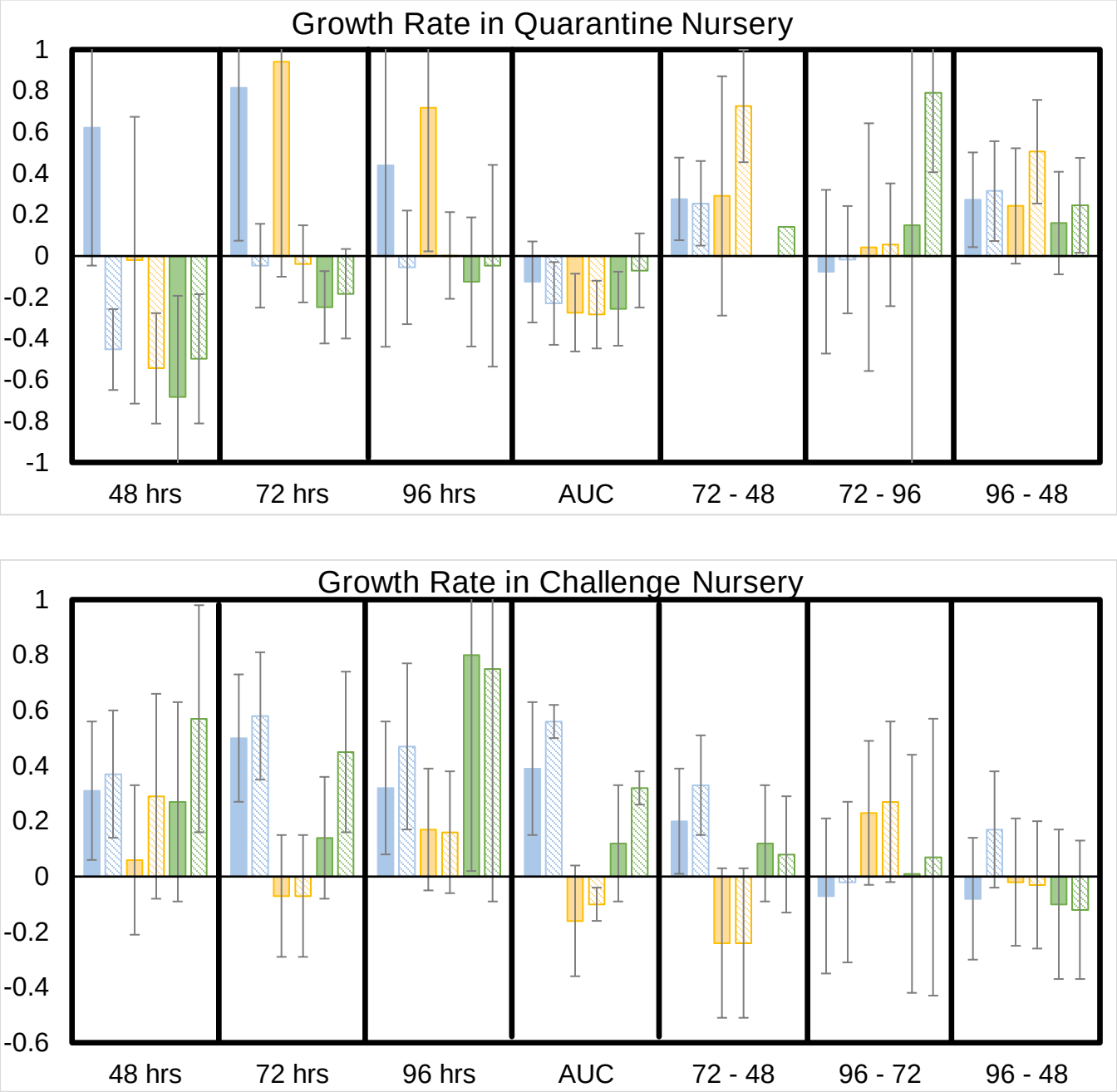
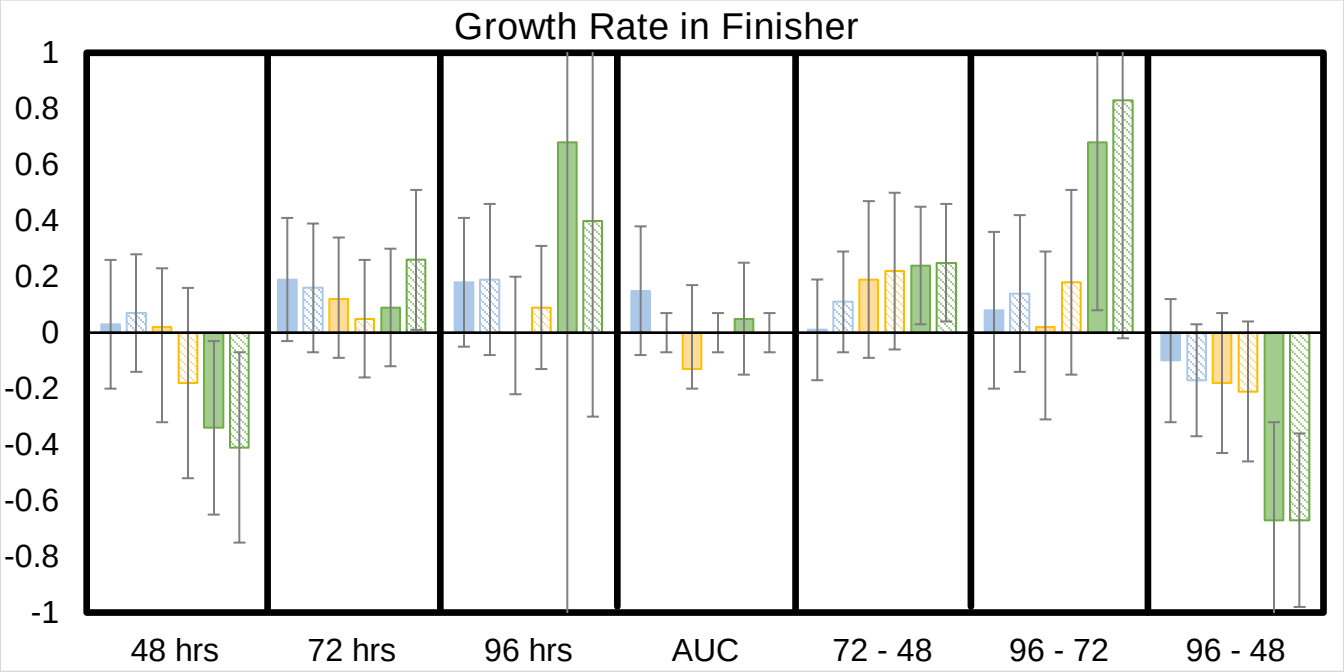


Figure 4.1: Estimates of genetic correlations (and SE bars) of MSA phenotypes with disease resilience traits for Concanavalin A (blue), Phytohemagglutinin (yellow), and Phorbol Myristate Acetate (green), using BIS (solid bars) and stimulated mean (striped bars) MSA phenotypes.



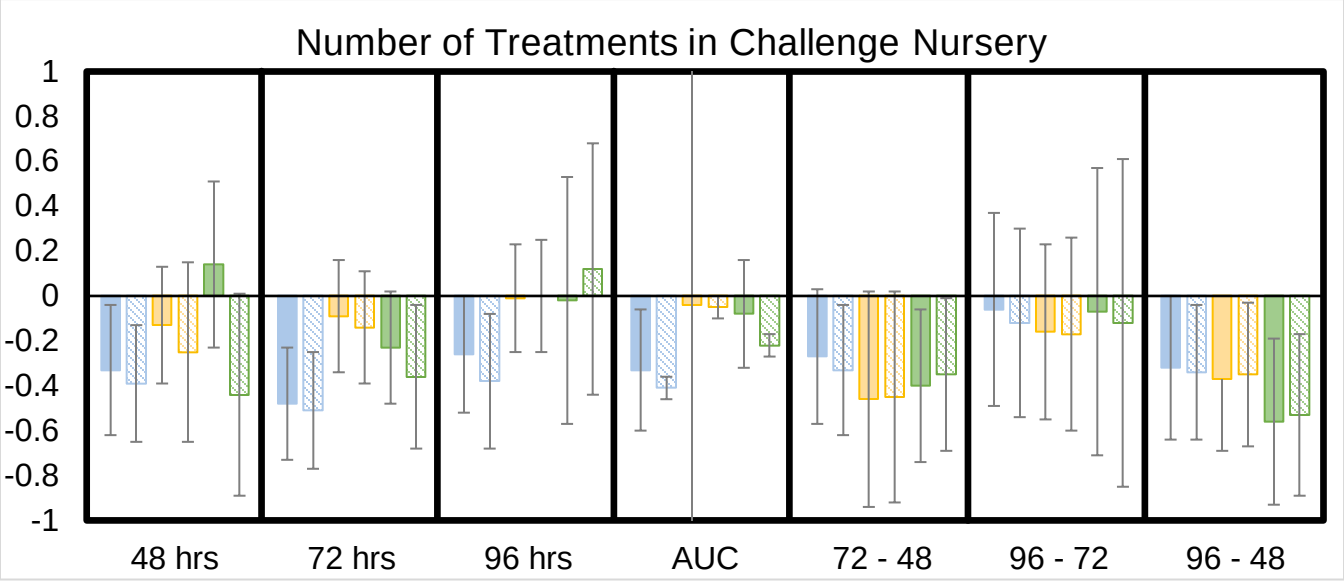
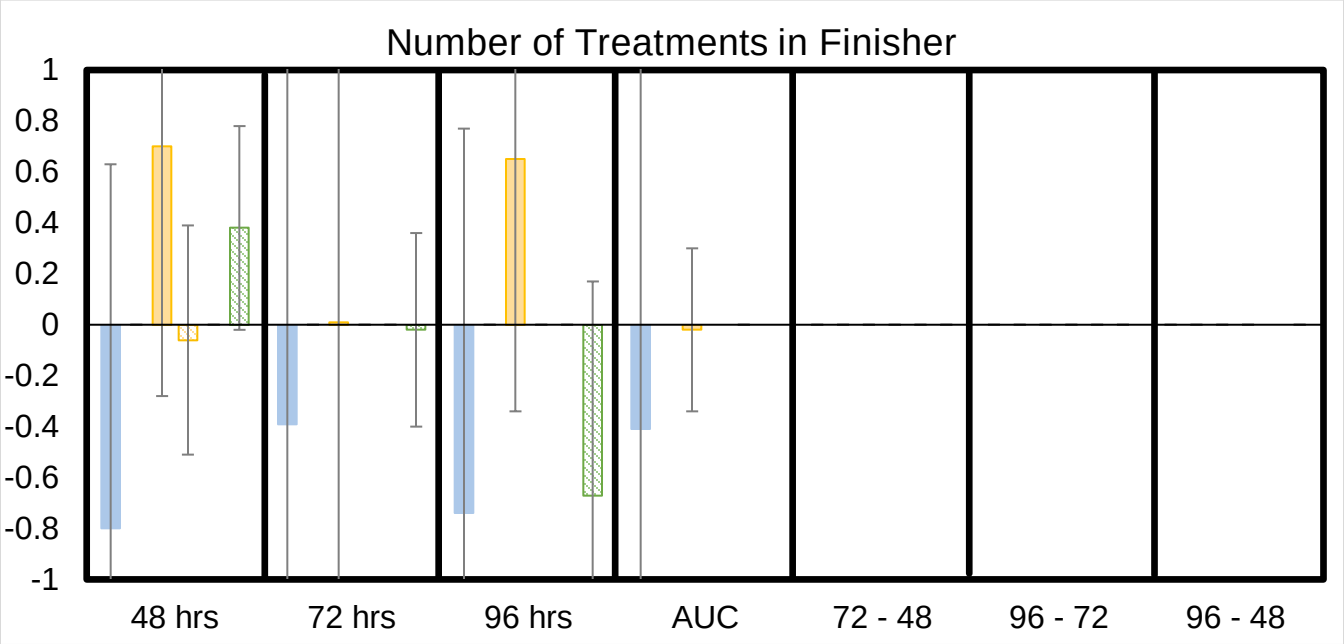


Figure 4.1: (continued)



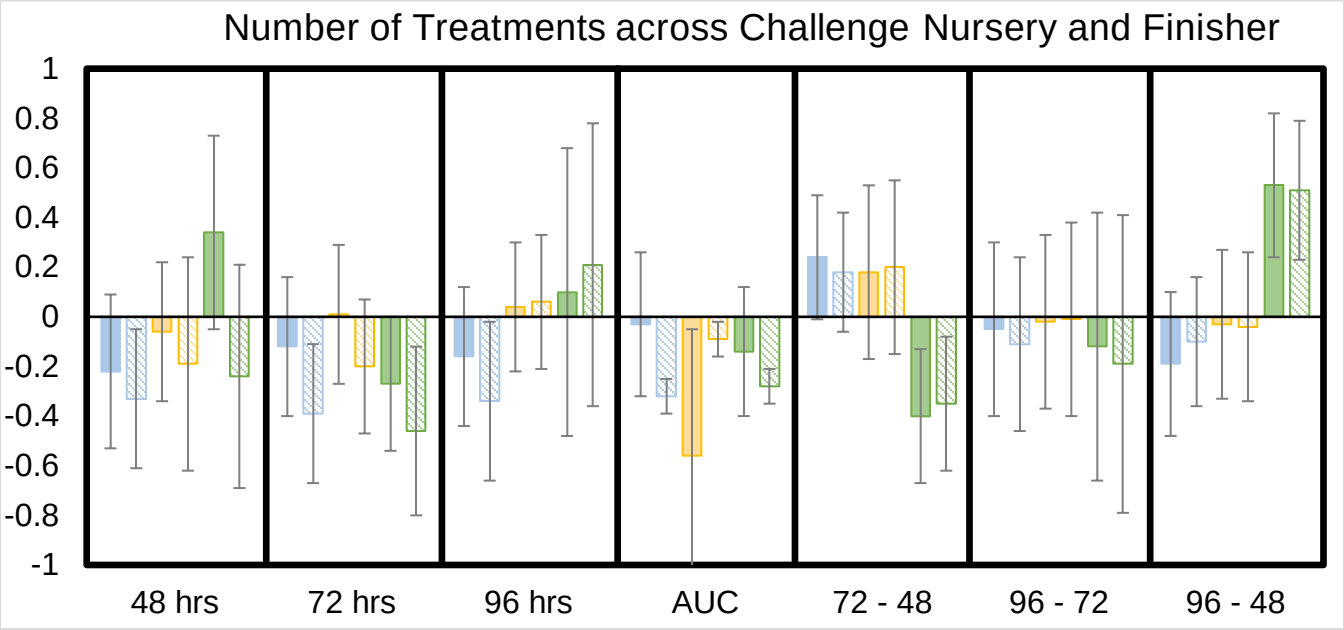
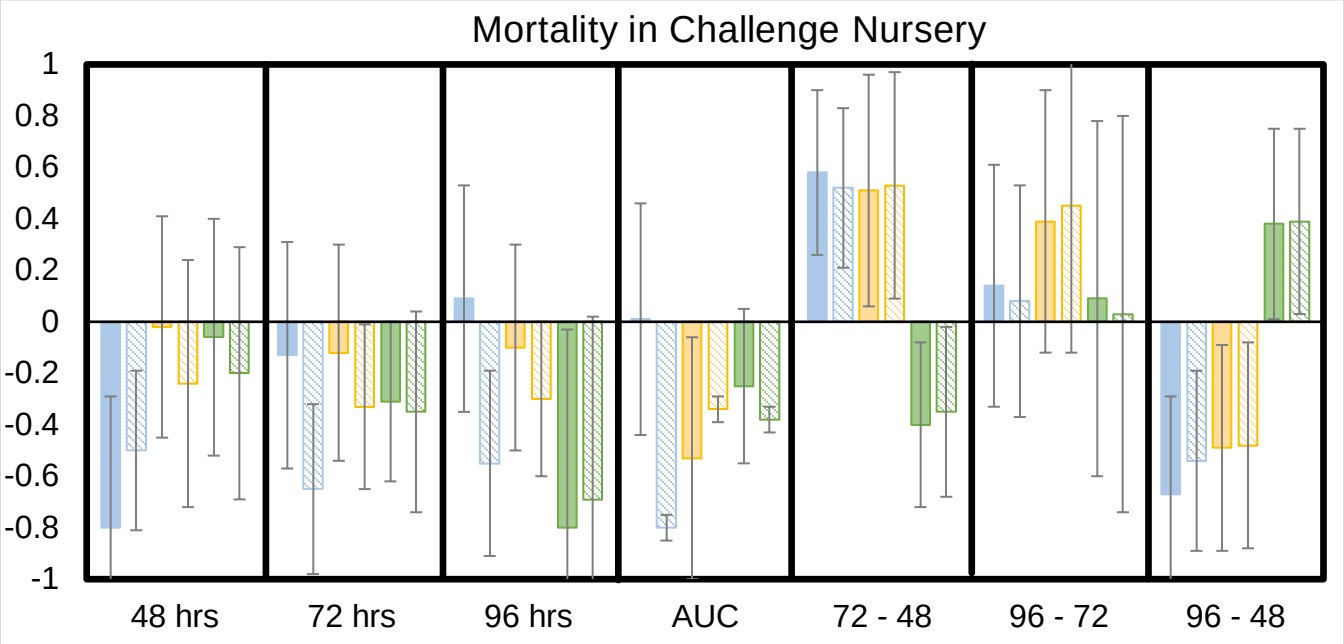


Figure 4.1: (continued)



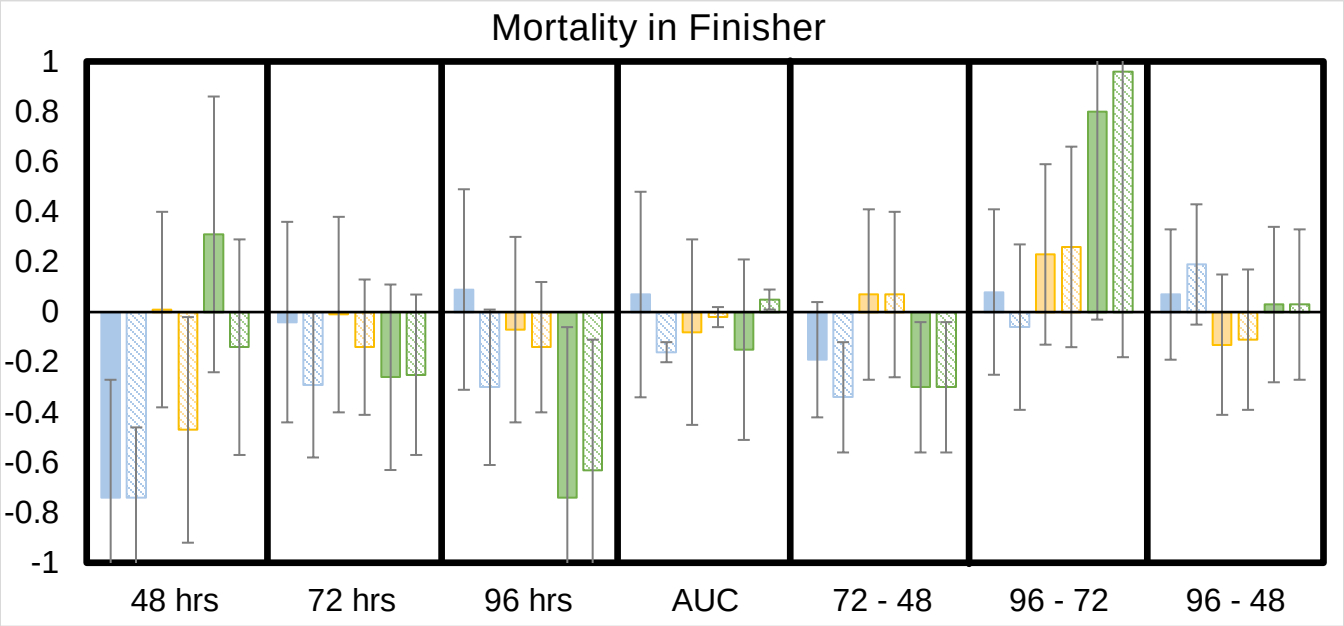
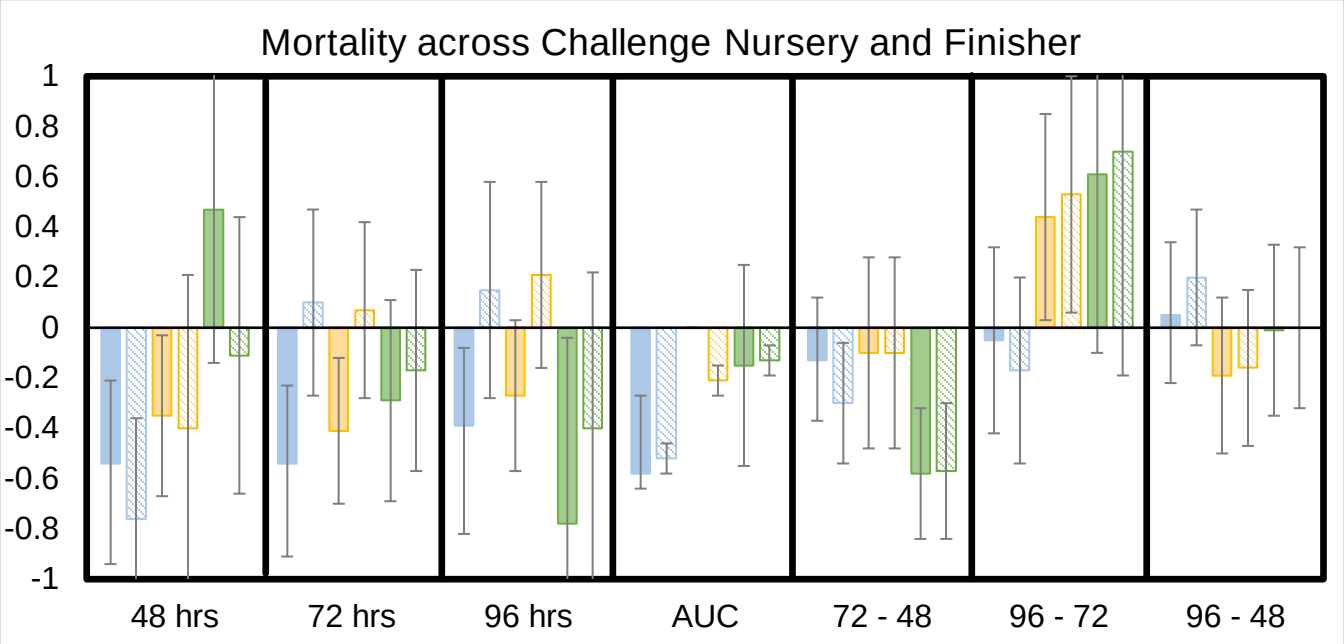


Figure 4.1: (continued)



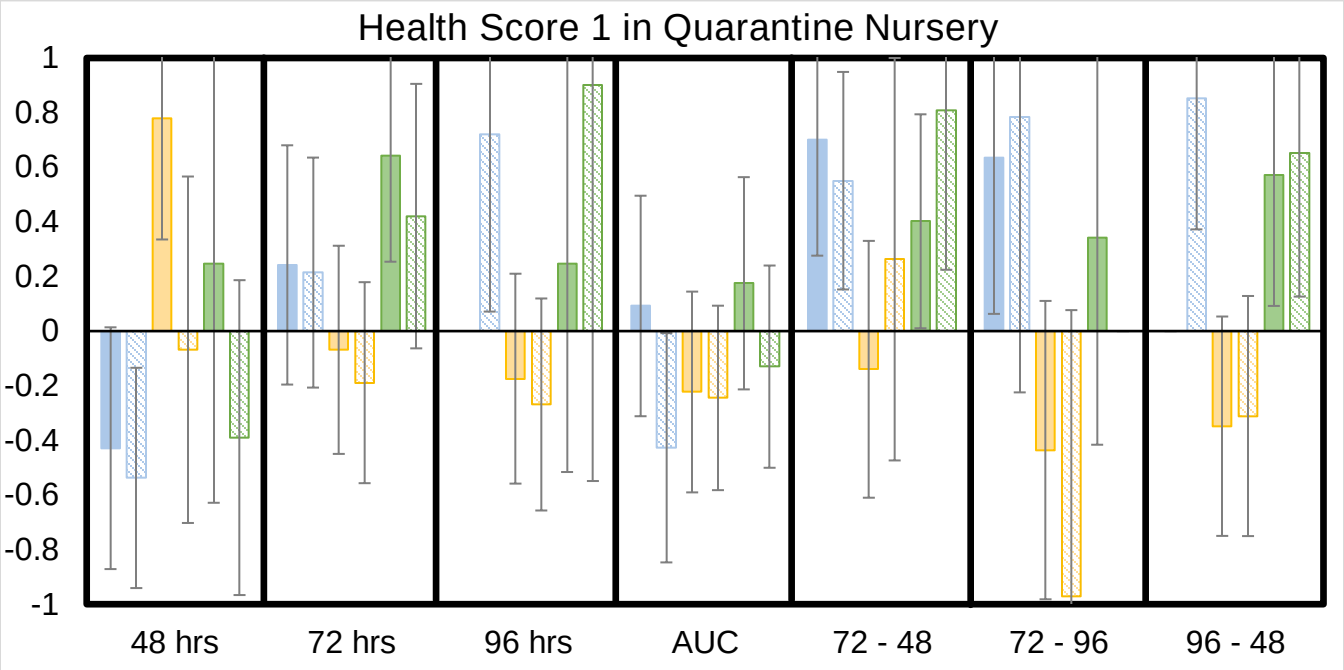
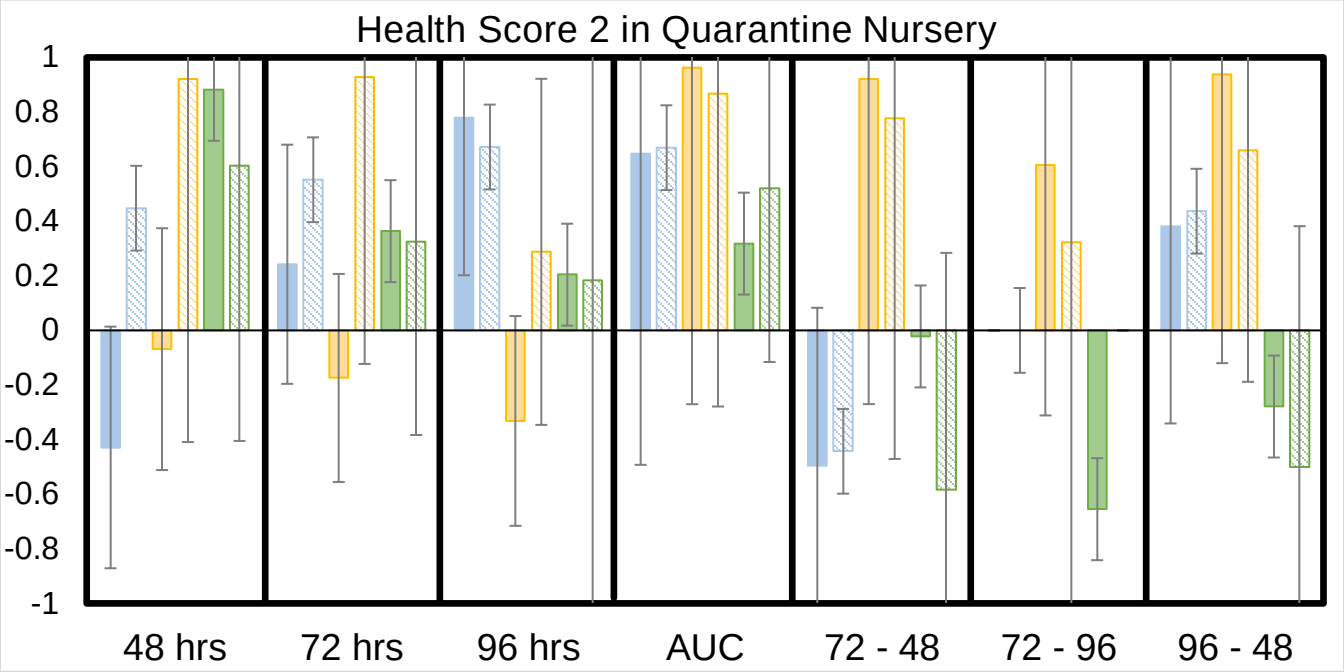


Figure 4.1: (continued)



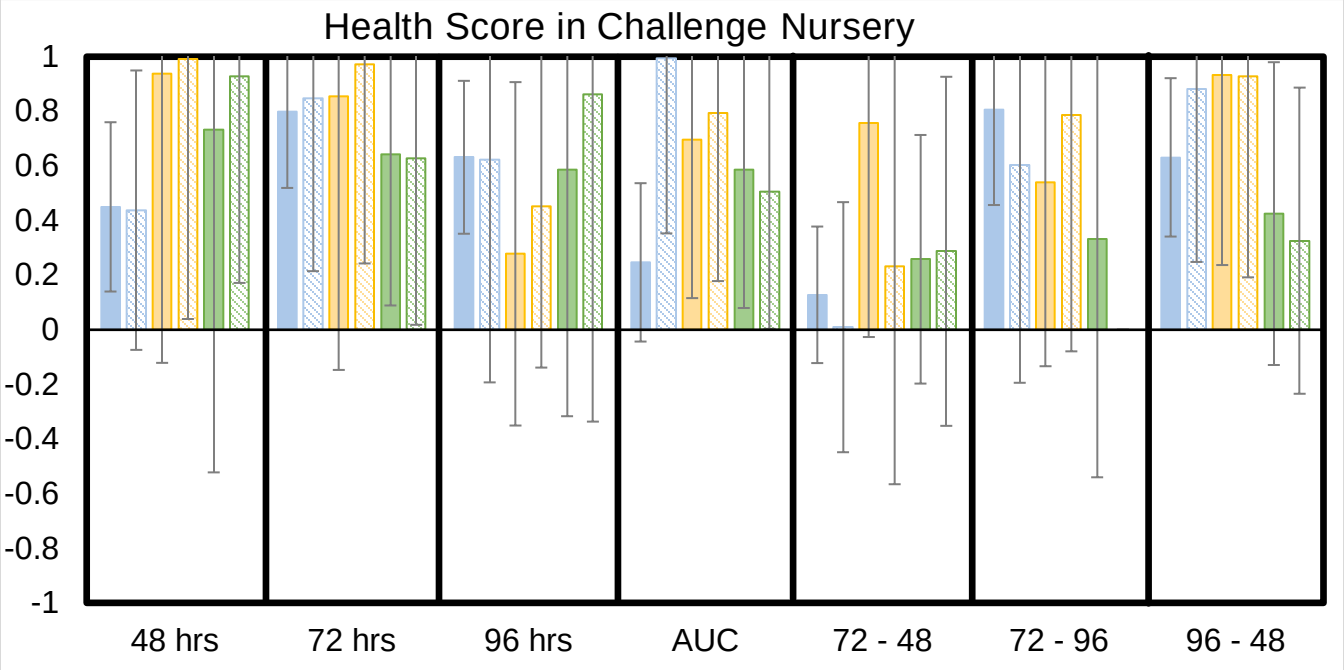


Figure 4.1: (continued)

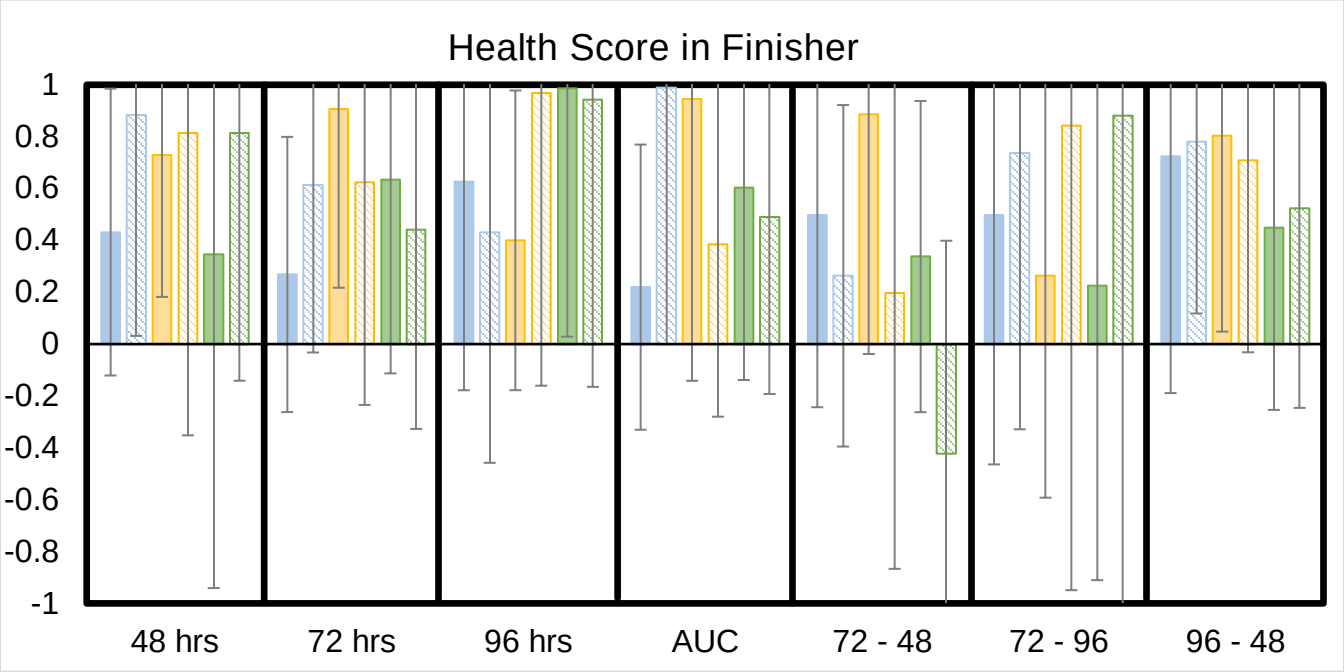


Figure 4.1: (continued)

Supplementary Table 4.1: Mitogen Concentrations used in the Mitogen Stimulation Assays.

Mitogen	Chemical	solution	[stock]	Cie	#	[4X]	[final]
A	Concanavalin A	con A	2 mg/mL	Sigma	SLBR2953V	20 μg/mL	5 μg/mL
B	Phytohemagglutinin	PHA-L	1 mg/mL	Sigma	SLBJ2065V	4 μg/mL	1 μg/mL
C	Pokeweed Mitogen	PWM	1 mg/mL	Sigma	SLBB6519V	10 μg/mL	2.5 μg/mL
D	Lipopolysaccharide	LPS	4 mg/mL	Invivo Gen	13106-MM	4 μg/mL	1 μg/mL
	Dextran Sulfate	DxS	400 μg/mL	Sigma	BCBN6770V	4 μg/mL	1 μg/mL
E	Phorbol 12-Myristate 13-Acetate	PMA	100 μg/mL	Sigma	D00145003	40 ng/mL	10 ng/mL
	Ionomycin	Iono	1 mg/mL	Sigma	GBC18082012	1 μg/mL	250 ng/mL

Supplementary Table 4.2: Estimates of phenotypic correlations for BIS (below diagonal) and Stimulated Means (above diagonal) (SE in parentheses) between time points (48, 72, or 72 hrs after stimulation) and for area under the curve (AUC) for each of five mitogens.

Concanavalin A	48	72	96	AUC
48		0.53 (0.03)	0.50 (0.04)	0.77 (0.02)
72	0.44 (0.03)		0.02 (0.05)	NE
96	0.47 (0.03)	0.66 (0.02)		0.66 (0.02)
AUC	0.61 (0.02)	NE	0.68 (0.02)	

Phytohemagglutinin	48	72	96	AUC
48		NE	0.48 (0.04)	NE
72	0.56 (0.02)		0.64 (0.03)	NE
96	0.53 (0.03)	0.63 (0.02)		0.63 (0.03)
AUC	0.65 (0.02)	NE	0.66 (0.02)	

Poke Weed Mitogen	48	72	96	AUC
48		0.44 (0.04)	0.07 (0.05)	0.65 (0.03)
72	NE		0.43 (0.04)	0.02 (0.06)
96	NE	0.31 (0.04)		NE
AUC	NE	NE	NE	

Lipopolysaccharide	48	72	96	AUC
48		0.39 (0.04)	0.31 (0.04)	0.71 (0.03)
72	NE		0.38 (0.04)	0.01 (0.06)
96	NE	NE		0.33 (0.04)
AUC	NE	NE	0.89 (0.56)	

Phorbol Myristate Acetate	48	72	96	AUC
48		0.54 (0.03)	0.49 (0.04)	NE
72	0.52 (0.03)		NE	0.11 (0.08)
96	0.39 (0.03)	NE		0.48 (0.03)
AUC	0.68 (0.02)	NE	NE	

Supplementary Table 4.3: Estimates of phenotypic correlations for BIS (below diagonal) and Stimulated Mean (above diagonal) data (SE in parentheses) between mitogens at a given time point (48, 72, or 72 hrs after stimulation) and for area under the curve (AUC)

Trait	Con A 48	PHA 48	PWM 48	LPS 48	PMA 48
Con A 48		0.56 (0.03)	NE	0.36 (0.04)	NE
PHA 48	0.51 (0.03)		NE	0.35 (0.04)	0.35 (0.04)
PWM 48	NE	NE		0.42 (0.04)	0.56 (0.00)
LPS 48	NE	NE	NE		0.33 (0.04)
PMA 48	NE	0.36 (0.03)	NE	NE	

Trait	Con A 72	PHA 72	PWM 72	LPS 72	PMA 72
Con A 72		0.66 (0.03)	0.47 (0.03)	0.27 (0.04)	0.51 (0.03)
PHA 72	0.61 (0.02)		0.51 (0.03)	0.27 (0.04)	0.51 (0.03)
PWM 72	0.43 (0.03)	0.41 (0.03)		NE	NE
LPS 72	0.08 (0.04)	NE	NE		0.23 (0.04)
PMA 72	0.51 (0.03)	0.56 (0.02)	0.40 (0.03)	0.10 (0.04)	

Trait	Con A 96	PHA 96	PWM 96	LPS 96	PMA 96
Con A 96		0.64 (0.03)	0.50 (0.03)	0.21 (0.04)	NE
PHA 96	0.64 (0.02)		0.45 (0.04)	NE	NE
PWM 96	0.44 (0.03)	0.43 (0.03)		0.24 (0.04)	0.51 (0.03)
LPS 96	0.05 (0.04)	0.05 (0.04)	NE		NE
PMA 96	NE	0.41 (0.03)	NE	NE	

Trait	Con A AUC	PHA AUC	PWM AUC	LPS AUC	PMA AUC
Con A AUC		0.72 (0.02)	0.58 (0.03)	0.30 (0.04)	0.61 (0.03)
PHA AUC	0.63 (0.02)		0.65 (0.03)	0.36 (0.04)	0.62 (0.03)
PWM AUC	0.46 (0.03)	NE		0.35 (0.04)	0.95 (1.59)
LPS AUC	0.10 (0.04)	0.00 (0.04)	NE		0.26 (0.04)
PWM AUC	0.54 (0.03)	0.58 (0.02)	NE	NE	

Supplementary Table 4.4: Frequency of treatment and mortality reasons

Treatment Reason	Count	Mortality Reason	Count
Respiratory distress (Thumping)	1369	Poor/skinny/hairy/Failure to thrive	289
Grey/Brown Scours	956	Thumping/Heavy breathing	187
Coughing	555	Sudden death	114
Lameness	449	Slaughter house	100
Yellow Scours (Salmonella)	383	Meningitis	61
Arthritis (swollen joint)	372	Arrival	50
Failure to Thrive/Poor/Skinny/Hairy	294	Lameness/arthritis	28
Meningitis (Convulsions)	95	Sampling	25
Conjunctivitis	94	Generalized weakness	12
Red Scours (Dysentry)	86	Yellow Scours (Salmonella)	10
Inappetance	83	Runt/ Cull	10
Greasy pig disease	63	Grey/Brown Scours	9
Erysipelas	32	Ataxia	7
Ataxia	21	Hernia	6
Fever	7	Scours	4
Preripheral cyanosis	5	Fracture/sprain	4
Rectal prolapse	3	Inappetance	4
Tail/Ear/Flank biting	1	Rectal prolapse	4
Other	34	Fighting	3
		Greasy pig disease	2
		Paralysed	2
		Stressed	2
		White (anemia)	2
		Bleeding	1
		Peripheral cyanosis	1
		Tail/Ear/Flank biting	1
		Intestinal torsion	1
		Other	34

Supplementary Table 4.5: Estimates of phenotypic correlations (SE in parentheses) of MSA phenotypes with disease resilience traits for BIS.

	Survivor									Expanded		
Trait	qNurADG	cNurADG 2	FinADG	NurTRT	FinTRT	AllTRT	NurMOR	FinMOR	AllMOR	FinADG	FinTRT	AllMOR
Con A 48	-0.11 (0.04)	0.07 (0.04)	-0.03 (0.05)	-0.03 (0.04)	-0.04 (0.05)	0.00 (0.04)	0.00 (0.04)	-0.01 (0.04)	0.00 (0.04)	-0.02 (0.05)	NE	-0.27 (0.29)
Con A 72	-0.05 (0.04)	0.12 (0.04)	0.00 (0.05)	-0.05 (0.04)	-0.01 (0.05)	-0.03 (0.04)	0.02 (0.04)	0.02 (0.04)	-0.05 (0.04)	-0.01 (0.05)	NE	-0.02 (0.04)
Con A 96	-0.03 (0.04)	0.05 (0.04)	-0.01 (0.05)	0.01 (0.04)	-0.08 (0.05)	-0.01 (0.04)	-0.05 (0.04)	-0.04 (0.04)	-0.02 (0.04)	0.01 (0.05)	NE	-0.02 (0.04)
Con A AUC	-0.08 (0.04)	0.11 (0.04)	-0.01 (0.05)	-0.04 (0.04)	-0.03 (0.05)	-0.02 (0.04)	0.01 (0.04)	0.00 (0.04)	-0.04 (0.04)	0.00 (0.01)	NE	0.00 (0.07)
72-48	0.05 (0.04)	-0.06 (0.04)	0.00 (0.05)	0.04 (0.04)	NE	0.00 (0.04)	-0.03 (0.04)	0.00 (0.04)	0.03 (0.04)	0.02 (0.05)	NE	-0.05 (0.04)
96-48	0.00 (0.04)	0.04 (0.04)	0.00 (0.05)	-0.03 (0.04)	NE	0.02 (0.04)	0.06 (0.04)	-0.05 (0.04)	-0.02 (0.04)	0.00 (0.04)	NE	0.04 (0.04)
96-72	0.05 (0.04)	0.01 (0.04)	0.02 (0.05)	-0.01 (0.04)	NE	-0.03 (0.04)	-0.04 (0.04)	0.03 (0.04)	-0.02 (0.04)	0.02 (0.05)	NE	0.00 (0.04)
PHA 48	-0.09 (0.04)	0.07 (0.04)	-0.06 (0.05)	-0.04 (0.04)	-0.07 (0.05)	-0.01 (0.04)	-0.07 (0.04)	-0.07 (0.04)	0.04 (0.04)	-0.06 (0.05)	NE	-0.02 (0.04)
PHA 72	-0.10 (0.04)	0.02 (0.04)	-0.03 (0.05)	0.03 (0.04)	0.05 (0.05)	0.02 (0.04)	-0.02 (0.04)	-0.02 (0.04)	0.03 (0.04)	-0.03 (0.05)	NE	0.00 (0.04)
PHA 96	-0.08 (0.04)	0.14 (0.04)	0.02 (0.05)	-0.04 (0.04)	-0.05 (0.05)	0.00 (0.04)	-0.01 (0.04)	-0.01 (0.04)	-0.04 (0.04)	0.02 (0.05)	NE	0.00 (0.04)
PHA AUC	-0.12 (0.04)	0.02 (0.04)	-0.02 (0.05)	0.02 (0.04)	0.02 (0.05)	0.02 (0.04)	-0.04 (0.04)	-0.04 (0.04)	0.04 (0.04)	0.04 (0.04)	NE	0.00 (0.04)
72-48	-0.03 (0.04)	0.02 (0.04)	-0.03 (0.05)	-0.02 (0.04)	NE	-0.04 (0.04)	-0.03 (0.04)	-0.07 (0.04)	-0.03 (0.04)	0.02 (0.04)	NE	0.00 (0.04)
96-48	0.01 (0.04)	-0.13 (0.04)	-0.04 (0.05)	0.07 (0.04)	NE	0.05 (0.04)	0.00 (0.04)	0.06 (0.04)	0.03 (0.04)	0.04 (0.05)	NE	0.00 (0.04)
96-72	-0.03 (0.04)	0.09 (0.04)	0.06 (0.05)	-0.05 (0.04)	NE	-0.02 (0.04)	0.03 (0.04)	0.00 (0.04)	0.00 (0.04)	0.12 (0.05)	NE	0.00 (0.04)
PMA 48	-0.10 (0.04)	0.04 (0.04)	-0.03 (0.05)	-0.06 (0.04)	NE	0.01 (0.04)	0.02 (0.04)	-0.01 (0.04)	0.01 (0.04)	NE	NE	-0.07 (0.04)
PMA 72	-0.07 (0.04)	0.06 (0.04)	0.03 (0.05)	-0.04 (0.04)	NE	-0.02 (0.04)	-0.02 (0.04)	-0.01 (0.04)	-0.02 (0.04)	0.09 (0.05)	NE	-0.10 (0.04)
PMA 96	-0.06 (0.04)	0.02 (0.04)	-0.02 (0.04)	-0.04 (0.04)	NE	-0.03 (0.04)	-0.03 (0.04)	0.05 (0.04)	-0.02 (0.04)	0.07 (0.05)	NE	-0.13 (0.04)
PWM AUC	-0.08 (0.04)	0.06 (0.04)	0.01 (0.05)	-0.04 (0.04)	NE	-0.02 (0.04)	-0.01 (0.04)	-0.01 (0.04)	-0.01 (0.04)	0.03 (0.05)	NE	-0.01 (0.02)
72-48	0.02 (0.04)	-0.04 (0.04)	-0.04 (0.05)	0.00 (0.04)	NE	0.03 (0.04)	0.06 (0.04)	0.01 (0.04)	0.08 (0.04)	0.02 (0.05)	NE	-0.03 (0.04)
96-48	0.00 (0.04)	0.03 (0.04)	0.01 (0.05)	0.00 (0.04)	NE	0.04 (0.04)	-0.08 (0.04)	0.02 (0.04)	-0.04 (0.04)	NE	NE	NE
96-72	0.03 (0.04)	0.01 (0.04)	0.03 (0.05)	0.07 (0.04)	NE	-0.01 (0.04)	-0.02 (0.04)	-0.08 (0.04)	-0.09 (0.04)	NE	NE	NE
PWM 48	0.00 (0.01)	0.04 (0.04)	-0.06 (0.05)	-0.04 (0.04)	-0.03 (0.05)	-0.06 (0.04)	0.03 (0.04)	0.05 (0.04)	0.06 (0.04)	NE	NE	NE
PWM 72	0.06 (0.04)	0.03 (0.04)	0.07 (0.05)	-0.06 (0.04)	0.03 (0.05)	-0.09 (0.04)	-0.02 (0.04)	-0.02 (0.04)	-0.02 (0.04)	-0.01 (0.05)	NE	-0.01 (0.04)
PWM 96	-0.03 (0.04)	0.04 (0.04)	0.05 (0.05)	-0.07 (0.04)	-0.06 (0.05)	-0.09 (0.04)	-0.03 (0.04)	-0.02 (0.04)	0.00 (0.04)	0.02 (0.05)	NE	0.02 (0.04)
PWM AUC	0.01 (0.04)	0.03 (0.04)	0.06 (0.05)	-0.06 (0.04)	0.01 (0.05)	-0.10 (0.04)	-0.03 (0.04)	-0.03 (0.04)	0.01 (0.04)	0.04 (0.02)	NE	-0.01 (0.03)
72-48	0.13 (0.04)	-0.02 (0.04)	-0.12 (0.05)	0.06 (0.04)	NE	0.05 (0.04)	0.01 (0.04)	0.01 (0.04)	0.06 (0.04)	NE	NE	NE
96-48	-0.27 (0.00)	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
96-72	0.04 (0.04)	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
LPS 48	-0.07 (0.04)	NE	NE	NE	NE	NE	NE	NE	NE	-0.03 (0.05)	NE	-0.04 (0.04)
LPS 72	-0.09 (0.04)	0.03 (0.04)	0.02 (0.05)	0.00 (0.04)	0.10 (0.05)	-0.04 (0.04)	0.00 (0.04)	0.00 (0.04)	0.00 (0.04)	0.05 (0.02)	NE	0.04 (0.10)
LPS 96	-0.04 (0.04)	0.00 (0.04)	0.03 (0.05)	-0.01 (0.04)	-0.06 (0.05)	0.01 (0.04)	-0.02 (0.04)	0.00 (0.04)	-0.02 (0.04)	0.02 (0.05)	NE	-0.10 (0.04)
LPS AUC	-0.10 (0.04)	0.04 (0.04)	0.00 (0.05)	-0.02 (0.04)	-0.02 (0.04)	-0.02 (0.04)	0.02 (0.05)	0.02 (0.04)	0.02 (0.04)	0.00 (0.05)	NE	-0.12 (0.04)
72-48		NE	NE	NE	NE	NE	NE	NE	NE	0.12 (0.04)	NE	-0.06 (0.04)
96-48	0.05 (0.04)	NE	NE	NE	NE	NE	NE	NE	NE	N/W	NE	-0.04 (0.04)
96-72	0.03 (0.04)	NE	NE	NE	NE	NE	NE	NE	NE	0.04 (0.04)	NE	-0.10 (0.04)

Survivor: data includes only pigs that reached slaughter; Expanded: data includes imputed and expanded phenotypes for pigs that died but that were included in the analyses with associate weight greater than 0.75; qNurADG: average daily gain in quarantine nursery; cNurADG: average daily gain in challenge nursery; FinADG: average daily gain in finisher; NurTRT: number of treatments per pig in challenge nursery; FinTRT: number of treatments per pig in finisher; ALLTRT: number of treatments per pig in challenge nursery and finisher; NurMOR: mortality rate for pigs in challenge nursery; FinMOR: mortality rate for pigs in finisher; ALLMOR: mortality rate for pigs in challenge nursery and finisher; Con A: Concanavalin A; PHA: Phytohemagglutinin; PWM: Poke Weed Mitogen; LPS: Lipopolysaccharide; PMA: Phorbol Myristate Acetate; AUC: Area Under the Curve; 48: 48 hrs; 72: 72 hrs; 96: 96 hrs; 72 – 48: difference between 72 hrs and 48 hrs; 96 – 72: difference between 96 hrs and 72 hrs; 96 – 48: difference between 96 and 48 hrs.

Supplementary Table 4.6: Estimates of phenotypic correlations (SE in parentheses) of MSA phenotypes with disease resilience traits for Stimulated Mean.

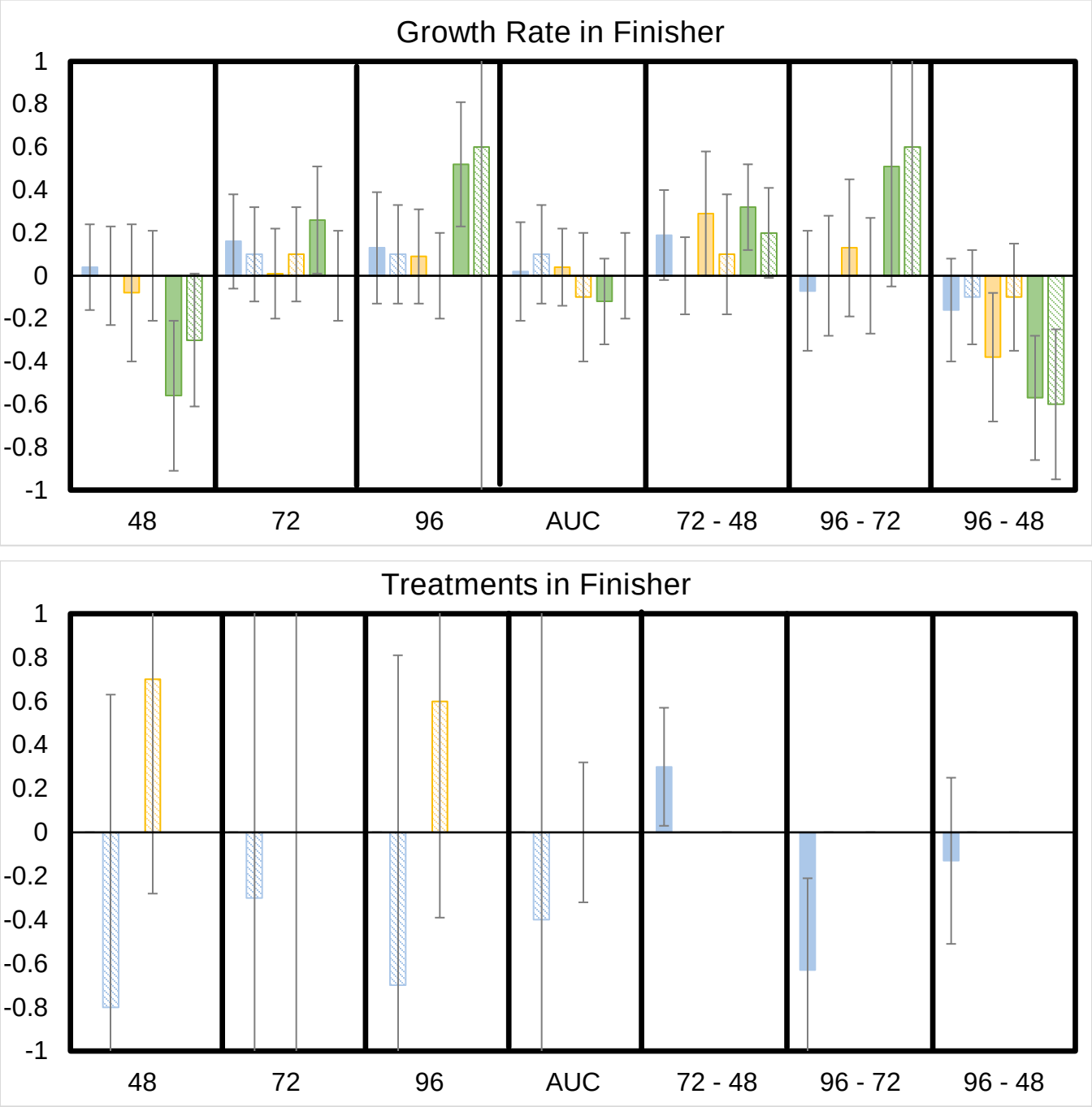
Trait	qNurADG	cNurADG	FinADG	NurTRT	FinTRT	AllTRT	NurMOR	FinMOR	AllMOR
Con A 48	-0.12 (0.04)	0.07 (0.03)	-0.01 (0.03)	-0.11 (0.10)	NE	-0.04 (0.12)	-0.03 (0.06)	-0.04 (0.08)	-0.02 (0.08)
Con A 72	-0.05 (0.04)	0.10 (0.03)	0.00 (0.03)	-0.10 (0.10)	NE	-0.11 (0.11)	-0.09 (0.06)	-0.06 (0.07)	0.02 (0.07)
Con A 96	-0.02 (0.04)	0.05 (0.03)	0.01 (0.03)	0.02 (0.10)	NE	-0.05 (0.11)	-0.02 (0.05)	-0.08 (0.07)	-0.09 (0.08)
Con A AUC	-0.08 (0.04)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
72-48	0.04 (0.04)	-0.09 (0.04)	-0.01 (0.05)	0.05 (0.04)	NE	0.00 (0.04)	-0.05 (0.04)	0.00 (0.04)	0.03 (0.04)
96-48	0.00 (0.04)	0.04 (0.04)	0.00 (0.05)	-0.03 (0.04)	NE	0.03 (0.04)	0.07 (0.04)	-0.04 (0.04)	-0.02 (0.04)
96-72	0.06 (0.04)	0.02 (0.04)	0.02 (0.05)	-0.01 (0.04)	NE	-0.04 (0.04)	-0.04 (0.04)	0.04 (0.04)	-0.01 (0.04)
PHA 48	-0.08 (0.04)	0.06 (0.04)	-0.07 (0.04)	-0.08 (0.10)	-0.18 (0.12)	-0.06 (0.12)	0.10 (0.07)	-0.04 (0.08)	-0.17 (0.10)
PHA 72	-0.09 (0.04)	0.03 (0.02)	-0.01 (0.02)	-0.01 (0.10)	-0.04 (0.14)	-0.07 (0.11)	0.01 (0.05)	-0.05 (0.06)	-0.07 (0.06)
PHA 96	-0.06 (0.04)	0.08 (0.02)	0.01 (0.02)	-0.12 (0.09)	-0.20 (0.15)	-0.07 (0.11)	-0.05 (0.04)	-0.08 (0.05)	-0.03 (0.05)
PHA AUC	-0.09 (0.04)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
72-48	-0.01 (0.04)	0.02 (0.04)	-0.04 (0.05)	-0.01 (0.04)	NE	-0.03 (0.04)	-0.03 (0.04)	-0.07 (0.04)	-0.03 (0.04)
96-48	0.02 (0.04)	-0.12 (0.04)	-0.05 (0.05)	0.07 (0.04)	NE	0.05 (0.04)	0.00 (0.04)	0.05 (0.04)	0.02 (0.04)
96-72	0.00 (0.04)	0.09 (0.04)	0.07 (0.05)	-0.06 (0.04)	NE	-0.02 (0.04)	0.03 (0.04)	0.00 (0.04)	0.00 (0.04)
PMA 48	-0.10 (0.04)	0.04 (0.04)	-0.02 (0.04)	-0.29 (0.11)	0.15 (0.12)	-0.21 (0.12)	-0.04 (0.07)	0.01 (0.08)	0.07 (0.09)
PMA 72	-0.06 (0.04)	0.06 (0.03)	0.05 (0.03)	-0.13 (0.10)	-0.04 (0.12)	-0.25 (0.12)	-0.04 (0.06)	-0.09 (0.08)	-0.08 (0.08)
PMA 96	-0.08 (0.04)	0.05 (0.05)	0.00 (0.04)	-0.18 (0.11)	-0.30 (0.15)	-0.34 (0.12)	0.07 (0.08)	-0.04 (0.08)	-0.14 (0.10)
PWM AUC	-0.11 (0.04)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.07)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
72-48	0.02 (0.04)	-0.05 (0.04)	-0.04 (0.05)	0.01 (0.04)	NE	0.03 (0.04)	0.05 (0.04)	0.00 (0.04)	0.08 (0.04)
96-48	-0.05 (0.04)	0.04 (0.04)	0.00 (0.05)	-0.07 (0.04)	NE	-0.03 (0.04)	0.02 (0.04)	0.00 (0.04)	0.04 (0.04)
96-72	0.00 (0.04)	0.02 (0.04	0.04 (0.05	0.05 (0.04	NE	-0.02 (0.04	-0.08 (0.04	-0.01 (0.04	0.00 (0.32
PWM 48	-0.01 (0.01)	0.00 (0.02)	0.02 (0.03)	-0.22 (0.10)	NE	-0.21 (0.12)	0.03 (0.05)	0.05 (0.06)	0.03 (0.06)
PWM 72	0.05 (0.04)	0.05 (0.03)	0.03 (0.03)	-0.20 (0.10)	NE	-0.22 (0.12)	-0.07 (0.06)	-0.07 (0.07)	-0.01 (0.08)
PWM 96	-0.06 (0.04)	0.03 (0.03)	0.02 (0.03)	-0.10 (0.10)	NE	-0.17 (0.12)	-0.04 (0.05)	-0.05 (0.07)	-0.02 (0.07)
PWM AUC	-0.01 (0.04)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	NE	0.00 (0.05)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
72-48	0.14 (0.04)	-0.02 (0.04)	-0.12 (0.05)	0.06 (0.04)	NE	0.05 (0.04)	0.01 (0.04)	0.01 (0.04)	0.06 (0.04)
96-48	-0.06 (0.03)	NE	NE	NE	NE	NE	NE	NE	NE
96-72	0.03 (0.04)	NE	NE	NE	NE	NE	NE	NE	NE
LPS 48	-0.07 (0.04)	0.02 (0.03)	0.02 (0.03)	-0.12 (0.11)	NE	-0.23 (0.12)	0.02 (0.06)	0.04 (0.07)	-0.08 (0.08)
LPS 72	-0.08 (0.04)	0.01 (0.03)	0.02 (0.04)	-0.03 (0.11)	NE	-0.10 (0.12)	0.04 (0.07)	0.08 (0.08)	0.07 (0.08)
LPS 96	-0.03 (0.04)	0.00 (0.04)	0.01 (0.04)	0.03 (0.05)	NE	0.08 (0.09)	-0.05 (0.08)	-0.09 (0.09)	-0.07 (0.10)
LPS AUC	-0.08 (0.04)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	NE	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
72-48	0.02 (0.04)	NE	NE	NE	NE	NE	NE	NE	NE
96-48	0.07 (0.16)	NE	NE	NE	NE	NE	NE	NE	NE
96-72	0.02 (0.04)	NE	NE	NE	NE	NE	NE	NE	NE

qNurADG: average daily gain in quarantine nursery; cNurADG: average daily gain in challenge nursery; FinADG: average daily gain in finisher; NurTRT: number of treatments per pig in challenge nursery; FinTRT: number of treatments per pig in finisher; ALLTRT: number of treatments per pig in challenge nursery and finisher; NurMOR: mortality rate for pigs in challenge nursery; FinMOR: mortality rate for pigs in finisher; ALLMOR: mortality rate for pigs in challenge nursery and finisher; Con A: Concanavalin A; PHA: Phytohemagglutinin; PWM: Poke Weed Mitogen; LPS: Lipopolysaccharide; PMA: Phorbol Myristate Acetate. AUC: Area Under the Curve; 48: 48 hrs; 72: 72 hrs; 96: 96 hrs; 72 – 48: difference between 72 hrs and 48 hrs; 96 – 72: difference between 96 hrs and 72 hrs; 96 – 48: difference between 96 and 48 hrs.

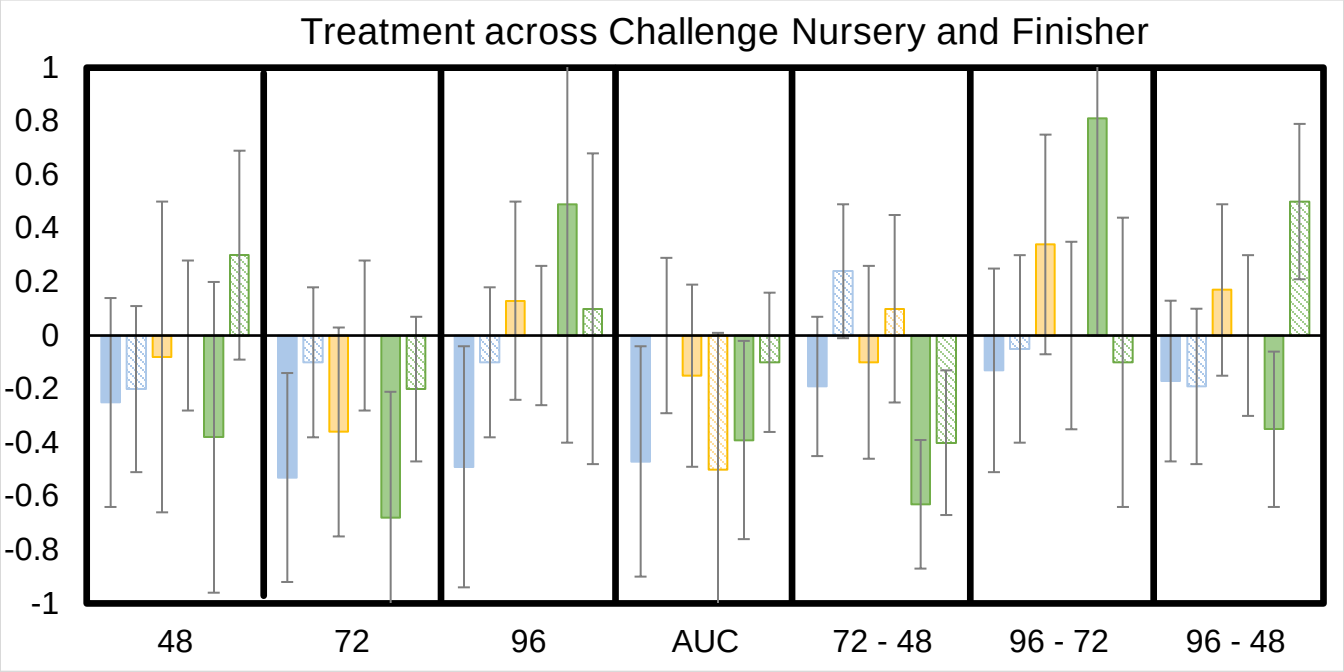
Supplementary Table 4.7: Estimates of phenotypic correlations (SE in parentheses) of MSA phenotypes with health score traits for both BIS and Stimulated Mean.

	BIS				Stimulated Mean			
Trait	qHScore 1	qHScore 2	cHScore	FinHScore	qHScore 1	qHScore 2	cHScore	FinHScore
Con A 48	0.10 (0.04)	-0.01 (0.04)	0.00 (0.04)	-0.04 (0.04)	0.08 (0.04)	0.01 (0.04)	0.02 (0.04)	-0.01 (0.04)
Con A 72	0.06 (0.04)	-0.05 (0.04)	-0.07 (0.04)	-0.05 (0.04)	0.06 (0.04)	-0.05 (0.04)	-0.05 (0.04)	-0.05 (0.04)
Con A 96	-0.01 (0.04)	0.01 (0.04)	-0.01 (0.04)	-0.03 (0.04)	-0.03 (0.04)	0.01 (0.04)	0.00 (0.04)	-0.03 (0.04)
Con A AUC	0.08 (0.04)	-0.05 (0.04)	-0.07 (0.04)	-0.06 (0.04)	0.05 (0.04)	-0.06 (0.04)	-0.03 (0.04)	-0.05 (0.04)
72-48	0.00 (0.04)	-0.07 (0.04)	-0.09 (0.04)	-0.04 (0.04)	-0.03 (0.04)	-0.09 (0.04)	-0.09 (0.04)	-0.05 (0.04)
96-72	-0.08 (0.04)	0.07 (0.04)	0.07 (0.04)	0.02 (0.04)	-0.08 (0.04)	0.07 (0.04)	0.08 (0.04)	0.03 (0.04)
96-48	-0.07 (0.04)	0.00 (0.04)	-0.03 (0.04)	-0.02 (0.04)	-0.10 (0.04)	0.00 (0.04)	-0.01 (0.04)	0.00 (0.04)
PHA 48	0.07 (0.04)	0.03 (0.04)	0.07 (0.04)	0.06 (0.04)	0.07 (0.04)	0.02 (0.04)	0.05 (0.04)	0.08 (0.04)
PHA 72	0.00 (0.04)	0.02 (0.04)	0.00 (0.04)	0.02 (0.04)	0.00 (0.04)	0.02 (0.04)	0.02 (0.04)	0.03 (0.04)
PHA 96	0.08 (0.04)	-0.04 (0.04)	-0.01 (0.04)	0.02 (0.04)	0.08 (0.04)	-0.04 (0.04)	0.00 (0.04)	0.02 (0.04)
PHA AUC	0.01 (0.05)	0.03 (0.04)	0.02 (0.04)	0.05 (0.04)	0.00 (0.04)	0.02 (0.04)	0.04 (0.04)	0.03 (0.04)
72-48	-0.05 (0.04)	0.00 (0.04)	-0.06 (0.04)	-0.03 (0.04)	-0.07 (0.04)	-0.02 (0.04)	-0.03 (0.04)	-0.02 (0.04)
96-72	0.09 (0.04)	-0.06 (0.04)	0.00 (0.04)	-0.01 (0.04)	0.08 (0.04)	-0.05 (0.04)	-0.01 (0.04)	-0.02 (0.04)
96-48	0.04 (0.04)	-0.05 (0.04)	-0.04 (0.04)	-0.02 (0.04)	0.02 (0.05)	-0.04 (0.04)	-0.01 (0.04)	-0.01 (0.05)
PMA 48	0.03 (0.04)	-0.06 (0.04)	-0.03 (0.04)	-0.03 (0.04)	0.03 (0.04)	-0.06 (0.04)	-0.03 (0.04)	-0.03 (0.04)
PMA 72	0.01 (0.04)	-0.07 (0.04)	-0.08 (0.04)	-0.06 (0.04)	0.00 (0.04)	-0.05 (0.04)	-0.05 (0.04)	-0.04 (0.04)
PMA 96	0.07 (0.04)	0.03 (0.04)	-0.04 (0.04)	-0.01 (0.04)	0.08 (0.04)	0.01 (0.04)	-0.05 (0.04)	-0.03 (0.04)
PWM AUC	0.02 (0.05)	-0.07 (0.04)	-0.07 (0.04)	-0.06 (0.04)	0.01 (0.04)	-0.05 (0.04)	-0.04 (0.04)	-0.04 (0.04)
72-48	-0.02 (0.04)	-0.01 (0.04)	-0.03 (0.04)	-0.03 (0.04)	-0.01 (0.04)	0.04 (0.04)	0.00 (0.04)	0.00 (0.04)
96-72	0.09 (0.05)	0.07 (0.04)	-0.01 (0.04)	0.03 (0.04)	0.08 (0.04)	0.03 (0.04)	0.02 (0.04)	0.01 (0.04)
96-48	0.03 (0.05)	0.07 (0.04)	-0.02 (0.04)	0.01 (0.05)	0.04 (0.04)	0.05 (0.04)	0.00 (0.04)	0.01 (0.04)
PWM 48	0.11 (0.04)	0.03 (0.04)	0.02 (0.04)	-0.01 (0.04)	0.00 (0.08)	-0.02 (0.04)	0.02 (0.04)	-0.02 (0.04)
PWM 72	0.06 (0.04)	-0.01 (0.04)	-0.04 (0.04)	-0.04 (0.04)	0.07 (0.04)	-0.01 (0.04)	-0.05 (0.04)	-0.04 (0.04)
PWM 96	0.03 (0.04)	0.00 (0.04)	-0.05 (0.04)	-0.05 (0.04)	0.03 (0.04)	0.01 (0.04)	-0.02 (0.04)	-0.02 (0.04)
PWM AUC	0.06 (0.05)	-0.07 (0.04)	-0.03 (0.04)	-0.05 (0.04)	0.08 (0.04)	-0.05 (0.04)	-0.03 (0.04)	-0.04 (0.04)
72-48	0.02 (0.02)	0.01 (0.04)	0.00 (0.04)	0.00 (0.04)	-0.03 (0.04)	0.00 (0.04)	-0.04 (0.04)	-0.02 (0.04)
96-72	-0.03 (0.04)	-0.01 (0.04)	-0.01 (0.04)	-0.01 (0.04)	-0.02 (0.04)	0.03 (0.04)	0.04 (0.04)	-0.01 (0.04)
96-48	-0.46 (0.04)	0.01 (0.04)	-0.06 (0.04)	0.03 (0.06)	-0.45 (0.02)	0.00 (0.05)	-0.05 (0.05)	-0.06 (0.05)
LPS 48	0.09 (0.04)	0.00 (0.04)	0.00 (0.04)	-0.05 (0.04)	0.09 (0.04)	0.00 (0.04)	0.00 (0.04)	-0.05 (0.04)
LPS 72	0.02 (0.04)	-0.05 (0.04)	0.01 (0.04)	0.00 (0.04)	0.01 (0.04)	-0.06 (0.04)	0.02 (0.04)	-0.01 (0.04)
LPS 96	-0.02 (0.04)	0.04 (0.04)	0.02 (0.04)	0.04 (0.04)	-0.03 (0.04)	0.03 (0.04)	0.03 (0.04)	0.04 (0.04)
LPS AUC	0.06 (0.05)	-0.05 (0.04)	0.00 (0.04)	-0.01 (0.04)	0.03 (0.04)	-0.05 (0.04)	0.00 (0.04)	-0.02 (0.04)
72-48	-0.05 (0.04)	-0.06 (0.04)	0.01 (0.04)	0.02 (0.04)	-0.09 (0.04)	-0.06 (0.04)	0.03 (0.04)	0.02 (0.05)
96-72	-0.04 (0.04)	0.09 (0.04)	0.02 (0.04)	0.05 (0.04)	-0.01 (0.04)	0.08 (0.04)	-0.01 (0.04)	0.02 (0.04)
96-48	-0.08 (0.04)	0.04 (0.04)	0.03 (0.04)	0.07 (0.04)	-0.09 (0.04)	0.05 (0.04)	0.05 (0.04)	0.08 (0.04)

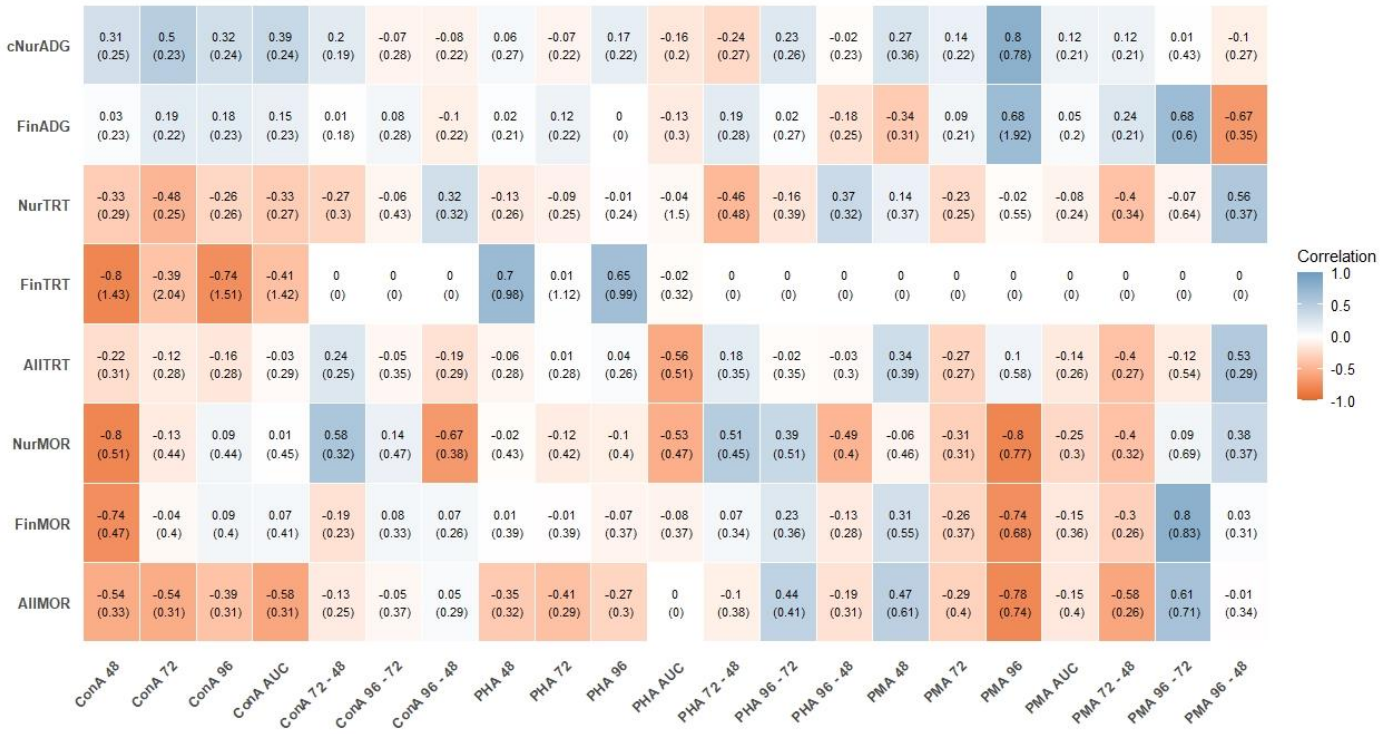
qHScore1: health score on the day of entry into the quarantine nursery; qHScore2: health score on two weeks after entry into the quarantine nursery; cHScore: health score on two weeks after entry into challenge nursery; FinHScore: health score on 6 weeks after entry into the finisher; Con A: Concanavalin A; PHA: Phytohemagglutinin; PWM: Poke Weed Mitogen; LPS: Lipopolysaccharide; PMA: Phorbol Myristate Acetate; AUC: Area Under the Curve; 48: 48 hrs; 72: 72 hrs; 96: 96 hrs; 72 – 48: difference between 72 hrs and 48 hrs; 96 – 72: difference between 96 hrs and 72 hrs; 96 – 48: difference between 96 and 48 hrs.



Supplementary Figure 4.1: Estimates of genetic correlations (and SE bars) of MSA phenotypes with disease resilience traits for Concanavalin A (Con A) in blue, Phytohemagglutinin (PMA) in yellow, and Phorbol Myristate Acetate (PMA) in green, using the Survivor (solid) and Expanded (striped) data sets.



Supplementary Figure 4.1 (continued)



Supplementary Figure 4.2: Heat map displaying estimates of genetic correlations (and SE) of MSA phenotypes with disease resilience traits for Concanavalin A (ConA), Phytohemagglutinin (PHA), and Phorbol Myristate Acetate (PMA).

cNurADG: average daily gain in challenge nursery; FinADG: average daily gain in finisher; NurTRT: number of treatments per pig in challenge nursery; FinTRT: number of treatments per pig in finisher; ALLTRT: number of treatments per pig in challenge nursery and finisher; NurMOR: mortality rate for pigs in challenge nursery; FinMOR: mortality rate for pigs in finisher; ALLMOR: mortality rate for pigs in challenge nursery and finisher; ConA: Concanavalin A; PHA: Phytohemagglutinin; PMA: Phorbol Myristate Acetate. AUC: Area Under the Curve; 48: 48 hrs; 72: 72 hrs; 96: 96 hrs; 72 – 48: difference between 72 hrs and 48 hrs; 96 – 72: difference between 96 hrs and 72 hrs; 96 – 48: difference between 96 and 48 hrs.

CHAPTER 5. DISCUSSION AND CONCLUSION

The objective of this thesis was to investigate the possibility of using SNP WUR10000125 (WUR) and Mitogen Stimulation Assays (MSAs) as possible genetic indicators to select for disease resilience in high-health nucleus breeding populations. Production losses due to infectious swine pathogens have a direct economic impact on the global swine industry and have serious implications for animal welfare, consumer trust, efficient livestock production, and for providing an efficient source of protein worldwide. Vaccines and medications are routinely prescribed to maintain herd health against various infectious swine pathogens, but studies indicate that for some diseases, such as PRRS, these preventative methods have minimal success. Additionally, there is growing consumer interest in animals that are raised without antibiotics, suggesting that genetic improvement for increased disease resilience that minimizes the use of medicated feeds and antibiotics can be a worthwhile strategy to implement in the swine industry. Since complete resistance to disease is rare, disease resilience is an economically valuable trait because it is defined as the ability for an animal to maintain performance during infection (Albers et al., 1987; Doeschl-Wilson et al., 2012; Mulder and Rashidi, 2017). Chapters 3 and 4 of this thesis investigated the potential of WUR and MSA as genetic indicators to select for increased disease resilience. The focus of this chapter is to discuss how these results can be used by swine breeders.

One global swine disease that has been heavily studied is PRRS. Prior to the NDCM, the PHGC was established to investigate the genetic basis of host response to PRRS in pigs. Based on the work of this consortium, several additional tools to combat PRRS coalesced as a result of collaborative efforts among various other institutions and the swine breeding industry. A genome wide association study determined that a region of *Sus scrofa* chromosome 4 (SSC4) had a large

effect on host response to PRRS and explained 15.7% of the genetic variance for PRRS viral load and 11.2% for weight gain after experimental infection of nursery pigs with the PRRS virus (Boddicker et al., 2012). Through functional genomics, it was later discovered that the likely cause of these effects was a splice site mutation in the GBP5 gene (Koltes et al., 2015). They also determined that genotype at the WUR10000125 SNP, which was on commercially available SNP panels, while the splice site mutation was not, was in complete linkage disequilibrium with genotype at the GBP5 SNP that caused the splice site mutation. However, a major limitation of the latter result was that the animal subjects were from one genetic line. In order to investigate the WUR SNP further, the relationship between the genotype at WUR with the genotype at its putative causative mutation in the GBP5 gene were reexamined in Chapter 3 using a larger sample size and pigs from a variety of breeds and family lines. Since the GBP5 splice site mutation is the putative causative mutation for the effect of this region on host response to PRRS (Koltes et al., 2015), another question addressed in Chapter 3 is whether the GBP5 marker has a larger association with host response to PRRS than the WUR marker. While these two objectives directed their attention to the PRRS virus, in practice, there are many more infectious pathogens than just the PRRS virus. Thus, the final research question answered in Chapter 3 was whether genotype at the WUR SNP is also associated with disease resilience to a natural polymicrobial disease challenge in the NDCM.

The great diversity of swine pathogens commonly found in swine barns creates a need for an efficient strategy to select for increased disease resilience. The main objective of Chapter 4 was to determine whether an in-vitro MSA on blood collected from young healthy pigs can be used as a genetic indicator for disease resilience to a polymicrobial disease challenge. In order for a genetic indicator to be effective, it must be heritable and have a considerable genetic correlation

with the trait of interest, in this case, disease resilience. The results presented in Chapter 4 estimated the genetic parameters of the MSA to determine if mitogen stimulation can be used as a suitable genetic indicator to select for increasing disease resilience.

The focus of this discussion is to expand on the results in these studies by discussing the implementation of WUR and MSA as genetic indicators for disease resilience, and the limitations of these two studies.

Implementation of WUR

In Chapter 3, it was found that the WUR and GBP5 SNPs are in close, but not perfect linkage disequilibrium (LD). We found that that the LD between these two SNPs the population of pigs we investigated was 0.94 contrary to the perfect LD that was estimated by Koltes et al. (2015). Differences between genotype at WUR and GBP5 SNP were determined to have resulted from genetic recombination and not from genotyping errors.

Using data from the PHGC trials, we found that, numerically, differences between genotypes were larger for the GBP5 SNP than for the WUR SNP for weight gain and viral load following experimental infection with the PRRS virus. This was as expected since the GBP5 SNP is the putative causative mutation for this major gene for host response to PRRS. In Chapter 3, we fitted both SNPs simultaneously to determine whether from adding the GBP5 SNP to a model that already included the WUR SNP explained a significant amount of extra variation. From this analysis it was determined that there was no statistical evidence to suggest that the GBP5 SNP was more strongly associated with host response to PRRS than the WUR SNP. On the SNP panels that were initially commercially available, the WUR SNP was included but the GBP5 SNP was not, and so the GBP5 SNP had to be genotyped separately in the PHGC trials.

This inconvenience thus raised the question if the WUR SNP is a suitable marker for the major QTL for host response to PRRS on SSC4. The GBP5 SNP has been included on more recent commercial SNP panels. However, since we determined that there was no statistical evidence suggesting that the effect of the GBP5 SNP is stronger than that of the WUR SNP, breeders can continue to use the WUR SNP and benefit from using either SNP to select for increased host response to PRRS.

A favorable response in disease resilience from selection for the favorable genotypes at the WUR SNP is expected, since it was found that, with the exception of two traits, all disease resilience, growth, carcass, and feed intake traits in the NDCM were favorably associated with the favorable genotype at the WUR SNP. However, the effect of WUR genotype was statistically significant ($p<0.05$) for only three traits: (1) average daily gain in the challenge nursery, (2) number of treatments in the challenge nursery, and (3) number of treatments across the challenge and finisher pens. These statistically significant differences suggest that by selecting based on the favorable allele of WUR, the offspring of selected individuals are expected to have a higher growth rate and lower treatments in the challenge nursery.

One strategy for implementing selection on genotype at the WUR SNP is by incorporating the WUR genotype into the selection index. Many breeding companies utilize an index that is composed of several traits that are given different weights to determine which animals would be the best selection candidates based on collected phenotypes. The WUR genotype can be included as another trait in the selection index since it was found that the favorable allele of WUR has favorable associations with many different traits. If financial constraints prevent phenotyping of animals in the candidate pool, then the genotype at the WUR

SNP can be used on its own as a tool to pre-select animals for further phenotyping, while increasing disease resilience.

The populations used in this study (PHGC and NDCM) were in Hardy Weinberg Equilibrium for the WUR SNP. This is surprising for two reasons: (1) this population did not undergo random mating, and (2) purebred lines were crossed, which intrinsically creates more heterozygotes in the population if the parental alleles differ in allele frequency. The frequency of the G allele in commercial lines is small (Boddicker et al., 2014b), which is likely because the selection is done at the high health nucleus level, where there tends to be little presence of the PRRS virus and, therefore, there has not been direct or indirect selection pressure on the G allele. The low frequency also suggests that it is possible to substantially increase the prevalence of pigs with the G allele. Thus, breeders can continue to use the genotype at the WUR marker as the criterion for selecting parents and implement marker-assisted selection for increased disease resilience in their breeding programs.

Implementation of MSA

The MSA phenotypes investigated in Chapter 4 provide other opportunities for phenotypes that can be measured on young healthy pigs in the nucleus. Selection on an indicator trait that is heritable and genetically correlated to the trait of interest is expected to result in a correlated response to selection in the target trait. Correlated response depends on the heritability of the indicator trait and the genetic correlation between the indicator and target trait. Based on the results in Chapter 4, MSA to Con A provides the most promising genetic indicator traits to select for disease resilience. Response to Con A had among the highest estimates of heritability at each of the three time points and for AUC, ranging from 0.23 to 0.27 when using BIS and

from 0.17 to 0.30 when using the stimulated mean. Responses to PHA and PMA had estimates of heritability in similar ranges but heritability estimates for PWM and LPS were consistently low. Thus, consideration was narrowed down to Con A, PHA, and PMA. When broadly analyzing the observed trends of the estimates of genetic correlations of these mitogens with disease resilience traits, Con A had the most consistent estimates relative to expected trends, which were that MSA phenotypes were positively correlated with growth rate under disease and negatively with numbers of treatments and mortality. While estimates of genetic correlations were largest for PMA for some disease resilience traits, these estimates also had rather large standard errors, whereas estimates for Con A had relatively smaller standard errors and, thus, were considered to be more reliable. Overall, all three time points following stimulation with Con A were promising, and more research is needed in order to determine which time point is best. Based on the moderate estimates of heritability at each of the three time points for Con A and expected trends in correlations with disease resilience traits, a correlated response to selection can be expected from selection on Con A MSA phenotypes to indirectly increase overall disease resilience of the swine herd.

Study Limitations

One limitation of the data from PHGC trials used in Chapter 3 is that the pigs were infected with only the North American strain of PRRS (NVSL). PRRS is recognized to be a multifaceted disease with high genetic variation, implying that multiple strains of PRRS exist and can evolve or appear in a short period of time. It is possible that the results obtained from the PHGC trials do not apply to all PRRS virus strains or could soon be outdated due to the fast evolution of the PRRS virus. It was found from the PHGC trials that there was a statistically

significant association between the WUR SNP genotype and PRRS viral load when using a North American strain of PRRS, NVSL-97-7895 (NVSL) (Boddicker et al., 2012). In a related study Hess et al. (2016) utilized a different North American strain, KS-2006-72109 (KS06) to investigate whether the WUR SNP was also associated with host response to PRRS in a genetically distinct North American strain of PRRS. This study determined that selection for host response to either of the genetically distinct strains is expected to increase host response to the other strain, implying that the effects of the two strains on host response to PRRS are similar.

One limitation of the natural disease challenge trials used in Chapter 4 is that all animals were offspring of a Landrace x Yorkshire cross, although from seven different genetic sources. Similar to how certain breeds of pigs have better meat quality or maternal ability, there is evidence suggesting that some breeds have higher resilience to disease than others. For example, Petry et al. (2005) found that Meishan and Large White pigs are more resistant to the effects of the PRRS virus than pigs of the Duroc and Pietrain breeds. In addition, the US swine industry does not exclusively use Landrace x Yorkshire crosses, as there are several other commonly used breeds used, such as the Duroc, Berkshire, or Hampshire breeds. While the effect of genotype at the WUR SNP was observed in many breeds commonly used in the swine industry (such as the Duroc, Pietran, and the Large White), this is not the case for studies performed using MSA. This suggests that further research should be done to determine if the genetic parameters of the MSA in other commonly used breeds are consistent with those presented in Chapter 4 in order to accurately predict the response to selection in different breeds.

Another limitation is that the results presented in Chapter 4 are based on in-vitro assays. There are several advantages to using an in-vitro assay to develop genetic indicator traits. One asset is that the mitogen does not need to applied to the pig directly, which would inherently

limit the use of each pig to just one mitogen. Additionally, this could lead to potential welfare problems, as the injection of a mitogen can cause significant pain or suffering to the animal. The collection of blood samples for in-vitro assays is much preferred for large scale projects such as those presented in Chapter 4, which consisted of thousands of pigs. One disadvantage of an in-vitro assay, however, is that it relies on the assumption that the wide range of different immune responses a pig may face can be captured by a single triggering mitogen. While a mitogen is effective in activating certain pathways of the immune response, it does so in a systematic fashion, akin to the body's response to a foreign antigen. It can be argued that this alone does not capture the diverse assortment of the body's response to a foreign pathogen. If this assumption is false, then the MSA provides valuable information about the animal's ability to activate a specific immune pathway, but not a specific immune response to any pathogen. Thus, any pathogen that would cause a response that involves a particular pathway would be correlated with the mitogen that stimulates the same pathway. More research should be done to investigate the genetic correlations of a mitogen with specific infectious pathogens that can provide more insight into the relationship between mitogen induced immune cell proliferation and disease.

The last limitation to be discussed is the limitation of the natural disease challenge used in both Chapter 3 and 4. Contrary to the PHGC trials, where each pig was injected with a given amount of PRRSV, the NDCM was a natural challenge, designed to emulate conditions of a high-pressure disease environment, such as those seen in a commercial swine barn. The NDCM's polymicrobial disease challenge consisted of over 30 different viruses, pathogens, and other infectious organisms. Pigs that were sick during the disease challenge were treated by veterinarians according to their observable symptoms. However, it was not determined which particular pathogen was the causative agent for any given illness. This leaves a gap in

information, as we were unable to narrow down our observations to any specific pathogen. For instance, many pigs required treatment due to respiratory illness. While PRRS is known to cause several respiratory symptoms, so can other diseases such as Swine Influenza and a myriad of other bacterial pathogens. Further research should be done to determine if there are genetic indicators that can be used specifically for just one disease. This may be useful for farms that fit a certain pathogenic profile, as not all farms will have the same pathogenic profile as the one used in the NDCM. While the pathogens used in the challenge are commonly found in North America, it would be useful to know if using a different combination of pathogens in a disease challenge will elicit different results than those presented in Chapters 3 and 4.

Concluding Remarks

The results presented in this thesis suggest that there not only is a substantial genetic component to a pig's response to PRRS and a polymicrobial disease challenge, but also that selection for increased disease resilience in high-health nucleus breeding populations is possible. Swine producers can benefit from marker-assisted selection by genotyping pigs at either the GBP5 or WUR SNP, as the favorable allele for both of these markers is in low frequency. Swine producers can also benefit from using Concanavalin A in a mitogen stimulation assay conducted on blood samples from young healthy pigs to indirectly select for increased disease resilience, as response to this mitogen not only was moderately heritable ($h^2 = 0.24$), but also had reasonable genetic correlations with all of the disease resilience traits collected in the polymicrobial natural challenge. Implementation of the WUR SNP or MSA for selection will not create pigs that are completely resistant to disease, but will reduce the financial costs of disease and allow breeders

an alternative to selection based on subjecting the nucleus herd to a disease challenge or collecting disease data at the commercial level.

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